CONTENTS OF VOLUME XIII

JANUARY, 1914 NUMBER 1

PAGE

STUDIES ON THE CIRCULATION IN MAN X THE BLOOD FLOW IN THE HANDS IN DISEASES OF THE HEART G N STEWART, M D, CLEVELAND 1

A STUDY OF THE BLOOD PRESSURE IN CHILDREN SHOWING ORTHOSTATIC ALBUMINURIA MURRAY H BASS, M D, AND H WISSLER, M D, NEW YORK 39

EXPERIMENTS TO DEVELOP A MORE WIDELY USEFUL METHOD OF BLOOD TRANSFUSION HENRY S SATTERLEE, M D, AND RANSOM S HOOKER M D, NEW YORK 51

THE CONDITION OF THE BLOOD IN HEMOPHILIA, THROMBOSIS AND PURPURA W H HOWELL, M D, BALTIMORE 76

A CLINICAL STUDY OF AMYLASE IN THE URINE, WITH SPECIAL REFERENCE TO THE PHENOLSULPHONEPHTHALEIN TEST H RAWLE GRIFFIN, M D, NEW YORK 96

THE CLINICAL STUDY OF HYPERCHOLESTERINEMIA HARRY B SCHMIDT M D, ANN ARBOR, MICH 121

THE UREA CONTENT OF THE SPINAL FLUID WITH SPECIAL REFERENCE TO ITS DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE A SERIES OF NINETY SEVEN CASES WILLARD E SOPER, M D, AND SELMA GRANAT, NEW YORK 131

THE GONOCOCCUS COMPLEMENT FIXATION TEST AND ANALYSIS OF RESULTS FROM ITS USE B A THOMAS, M D, AND ROBERT IVY, M D, PHILADELPHIA 147

A COMPARISON OF THE EXCRETORY POWER OF THE SKIN WITH THAT OF THE KIDNEY THROUGH A STUDY OF HUMAN SWEAT H W FRANCE MEYER, M D, AND E K MARSHALL, JR, PH D, BALTIMORE 159

STATUS LYMPHATICUS IN ADULTS ITS CLINICAL DIAGNOSIS AND IMPORTANCE HAVEN EMERSON, M D, NEW YORK 169

The Archives of Internal Medicine

Vol XIII JANUARY, 1914 No 1

STUDIES ON THE CIRCULATION IN MAN

X THE BLOOD-FLOW IN THE HANDS IN DISEASES OF THE HEART *

G N STEWART, M D
CLEVELAND, O

GENERAL REMARKS

It has been mentioned in the introduction to the last paper[1] that when proper precautions are taken there is a very fair degree of constancy in the results obtained in successive observations on different days on one and the same patient, provided gross changes in his clinical condition are excluded Accordingly, a marked change in the flow not accounted for by changes in the external conditions, especially in the external temperature, always suggests some decided alteration in the clinical state of the patient On the other hand, in certain cases, information may be obtained as to the state of the patient, for example, in regard to the lability of his vasomotor mechanism or the power of adaptation of his heart, by purposely changing the external conditions or by taking advantage of changes that have spontaneously occurred, in order to determine their effect on the flow in the hands or feet

In comparing the flows in the whole series of cases investigated with those in normal persons, one is struck by the fact that while the flow in persons suffering from well characterized pathological changes (compensated valvular lesions, for example) may be of the normal magnitude, having regard to the age of the patient, the room temperature, etc, in a much larger number of cases it is below the normal, often greatly below it Sometimes there is good evidence that this diminution in the hand or foot flow is the expression of an increased excitation of the vasoconstrictor mechanism as a whole or at least of the portion of it which presides over the cutaneous vessels not necessarily related to any material diminution in the driving power or the output of the heart In other cases there is good evidence that the diminished flow in the hand (or foot) is in the main the expression of diminished functional capacity on the part of the heart, associated either with engorgement of the veins

* From the H K Cushing Laboratory of Experimental Medicine, Western Reserve University

1 Paper IX of this series

and the right side of the heart or with diminution in the filling of the arteries without increased venous pressure In still other cases evidence may be obtained of a diminished flow in one or both hands or feet, which is solely or mainly a local condition A local increase in the flow may accompany such conditions as inflammation or vasomotor paralysis

The question whether the flow in the hands or feet gives any indication of the functional capacity of the heart would be best discussed on the basis of an experimental comparison of the heart output, as determined gasometrically, with the hand flow in normal and pathological cases Even in the absence of such a comparison, however, it would appear, that where the hand (or foot) flow is not less than normal it is only in exceptional conditions (local vasomotor paralysis of hands and feet?) that any gross error will be made in assuming that the heart is discharging an amount of blood not inferior to the normal When, however, the flow in the extremities is permanently and decidedly less than normal, several points have to be considered In many of these cases in which the normal ratio of the hand flow to the foot flow is preserved[2] in spite of the diminution in the flow and in which no anemia is present there is often good evidence that the lessened driving power of the heart is primarily responsible for the diminished flow On the other hand, in the anemias[3] the diminution of the flow in the hand and in the skin generally seems to be a compensating mechanism which permits an even increased quantity of blood to be sent by the heart through the lungs

A marked disturbance of the normal ratio between the hand and foot flow, of course gives no information as to the functional capacity of the heart, but indicates in the absence of local anatomical changes which might account for the difference (arteritis, paresis, etc), a change in vasomotor excitability Usually, this is manifested in a vasoconstriction of the feet, and a foot flow smaller than the normal in comparison to the hand flow Since the feet are normally but little exposed in comparison with the hands, local vasoconstriction connected with the technic of the measurements is more likely to occur in the feet than in the hands In various pathological conditions, for instance fever,[4] where the vasoconstrictor mechanism of the skin is abnormally excitable the tendency of vasoconstriction in the feet on exposure is intensified For this reason, as well as on account of the inferior flow per unit of volume in the feet, measurements of the hand flow are always to be preferred to measurements of the foot flow when the object is to obtain general information concerning the circulation and not to study local circulatory conditions A disturbance of the normal ratio between the hand and

2 See Paper VII of this series, Jour Exper Med 1913, xviii, No 1
3 Paper VI of this series, Jour Exper Med , 1913, xviii, No 2
4 See Paper VIII of this series, Jour of Exper Med , 1913, xviii No 1

foot flow, due not to vasomotor, but to anatomical causes (arteritis obliterans, for example), would be revealed by the fact that the ratio remains permanently abnormal even when the external conditions, the room temperature especially, are changed so as to abolish vasomotor differences of the lower and the upper extremities, or when this is done by the administration of vasodilator drugs

A few words are necessary in regard to the classification of the cases dealt with in this paper Some overlapping between the groups is unavoidable and the attempt has been made to assign each case presenting a complication of lesions to the group in which the factor seemingly predominant in the clinical picture would place it For example, in the group of myocardial affections, cases are necessarily included in which there were definite and demonstrable valvular lesions Thus certain cases with mitral stenosis (and insufficiency) are placed in the myocardial group because there is evidence that auricular fibrillation was also present, and that probably this condition with the consequent marked disturbance of the action of the ventricle was at the time of the investigation a more important factor in the clinical situation than the valvular lesion, or at least a factor which differentiated the case from one with the valvular lesion alone, and gave to it an added gravity Among the few cases classed as arteriosclerosis, a group which might, of course, have been greatly swollen at the expense of the other groups had attention been paid solely to this condition, is one[5] which might also have gone with the valvular lesions It is included with the cases of arteriosclerosis simply because that condition was extremely marked in the accessible arteries

I CONDITIONS IN WHICH SIGNS OF INVOLVEMENT OF THE MYOCARDIUM PREDOMINATED

There is probably no pathological condition of the heart in which such small hand flows are encountered as in this group While the myocardium is acting well, grave valvular defects may, as is well known be overcome and the output remain for long periods quite at the normal level The results of the blood-flow measurements in many cases of valvular lesions with good compensation are perfectly normal With a heart whose myocardium is seriously crippled even when the valves are anatomically and probably functionally intact, the hand flow may sink to astonishingly small figures Nor is this difficult to understand A pump with somewhat leaky valves may still deliver a good stream when the strokes are strong and regular No matter how tight the valves are the output will be small if the stroke is feeble uncertain and irregular.

5 Proc S Paper XI of this series

Thus in Stephen F, a man 31 years old, the flow in the hands was only 0 2 gram per 100 c c of hand per minute with a room temperature of 23 5 C increased to 0 5 gram in the right hand during immersion of the left in warm water, or not more than one-twentieth of the normal flow The protocol of this case has already been published [6]

The man, a contractor in a small way for brick and carpenter work, was examined in the dispensary and was admitted the same day to Lakeside Hospital where he died a few days later He stated that between three and four years previous to admission he became ill after a heavy lift and had never been well since All the signs of cardiac failure were present To the left the cardiac dulness extended almost to the anterior axillary line There was marked arhythmia with numerous premature contractions For some time past he had been aware of the irregular action of his heart and stated that it sometimes stopped for a beat The pulse-rate was only 60 (counted at the apex beat with the stethoscope) The neck veins were much engorged in the supine position There was edema of the feet and ankles The hands and feet were habitually cold although the weather was mild The whole surface was pale and cool There was no indication of valvular lesions No murmurs were detected during the time the patient lived The lungs were clear The clinical diagnosis was myocarditis without valvular lesion The immediate cause of death was pontine hemorrhage At autopsy the findings were fibrous myocarditis with relative insufficiency but no valvular lesion, pericarditis, perihepatitis and atrophic cirrhosis of the liver, and acute pulmonary edema

In Mrs A, a woman aged 72, who came to the dispensary complaining of palpitation of the heart and irregular pulse, the blood-flow in the left hand came out 0 78 gram per 100 c c per minute, and in the right 0 45 gram, with room temperature 22 5 C It must be taken into account that the hands in this case, being the typical hands of old age, consisted largely of bone which has a small blood-flow

The irregularity of the heart has been noticed for the past six years The heart sounds were clear and no murmurs could be detected, but there was marked arhythmia The pulse rate was 92 in sitting position There was no edema, no cough There was a history of rheumatism at 50 years of age, and again the winter before admission The hands were very thin and rather cool, although she wore gloves and said they were as warm as usual A diagnosis of senile heart with chronic myocarditis, arhythmia and arteriosclerosis was made She continued to come periodically to the dispensary for over four months, still complaining of palpitation although the pulse became more regular

The difference in the flow in the two hands (it must of course be noted that the accuracy of the measurements is less with these small flows than with normal flows) suggests that the smallness of the flow may be partly due to local vasoconstriction, which is often temporarily unequal for the two hands, and not altogether to the small output of the heart The question may indeed be raised whether as in the anemias a peripheral constriction may not occur in these cases in the interest of an increased flow through the lungs (and through the brain and other

6 Cleveland Med Jour, 1911 x, 385
7 Jour Exper Med, 1913, xviii, No 2

vital organs) In some ways the question is merely an academic one, since it is well known that in marked anemia the heart muscle may be impaired It is clear that the percentage content and the total absolute amount of hemoglobin in the blood being normal, there may still be oxygen deficiency if the weakened heart is unable to propel the normal amount of blood from the left ventricle through the systemic vessels to the right side of the heart On the supposition that the right ventricle could deal with a larger quantity of blood than is actually brought to it, a diminution in the average circulation time of the blood through the systemic path brought about by vasoconstriction confined to or predominating in the periphery might increase the minute volume of the heart In this case the small hand flow, where the myocardium is seriously damaged, would be due to two things First, diminished driving power of the heart and second, constriction of the vessels of the peripheral parts in excess of that which would correspond to the diminished filling of the vessels due to the deficient action of the heart alone Although the output in such cases as Stephen F is diminished, it is not conceivable that it is diminished in the same proportion as the hand flow

Jacob W, a house carpenter aged 59, height 5 feet, 1 inch, weight 173 pounds, was admitted at the dispensary April 4, 1911, suffering from myocarditis with anginal attacks Six months previously while going to church he was suddenly seized with substernal pain, with great dyspnea He had to sit down and could not speak Since that time he has had a spell every morning, lasting two to five minutes and it is getting worse If the pain is severe the left hand and forearm get dead Since yesterday he has had palpitation His general health was good until six months ago He had rheumatism eighteen years ago He used to drink beer and whisky moderately and to smoke moderately He denies lues Physical examination, cyanosis, some icterus, emphysema, heart generally enlarged, no murmurs The heart sounds are weak, blood-pressure high, some sclerosis of the arteries Urine negative

He came periodically to the dispensary after this April 24, 1911, his systolic blood pressure was 190, diastolic 110 April 27, 1911, he had five attacks during the night May 1, 1911, he had a mild attack while being examined in the dispensary The pain was felt right beneath the sternum and radiated down the inner side of the left arm and up the neck to the chin The pulse becomes somewhat smaller during the attack but the rate does not change The heart is enlarged 1 cm to the left but not to the right He is still stout He is being treated with potassium iodid and sodium nitrite The blood flow in the hands was examined May 1, 1911 Mouth temperature 37 1 C Pulse (sitting) 100, and rather small Hands in bath at 2 44 p m, in calorimeters at 2 55 * At 3 10 p m left hand put into water at 42 9 C (Table 1)

Cooling of calorimeters R, 0 11 degrees in fourteen and one-half minutes, Left 0 11 degrees in twenty-one and one half minutes Volume of right hand, 508 c c of left hand 486 c c The hands were fat

The flow in the right hand works out at 2 46 grams per 100 c c per minute, and in the left hand at 1 96 grams When the left hand was

* Three thousand and fifteen c c of water were put into each calorimeter In the protocols unless otherwise mentioned, this was the quantity always used for the hand calorimeters

immersed in warm water the flow in the right rose to 3 86 grams, a fair reflex vasodilatation Subsequent immersion of the left hand in cold water caused little change in the flow in the right

TABLE 1.—Calorimetric Measurements in Case of Jacob W

Time	Right	Left	Room Temp C	Time	Right	Notes
2 54	29 83	29 91	24 1	3 14	30 12	
2 56	29 81	29 86		3 15	30 14	
2 57	29 82	29 86		3 16	30 18	
2 58	29 84	29 87		3 17	30 20	Room 25 0 C
2 59	29 87	29 88	24 9	3 18	30 23	
3 00	29 87	29 89		3 19	30 26	
3 01	29 88	29 91		3 20	30 29	At 3 20 left hand put in water at 11 5 C
3 02	29 89	29 92		3 21	30 32	Room 25 1 C
3 03	29 89	29 92		3 22	30 34	
3 04	29 91	29 93	25 0	3 23	30 36	
3 05	29 92	29 94		3 24	30 39	
3 06	29 93	29 94		3 25	30 41	
3 07	29 95	29 95		3 26	30 44	
3 08	29 98	29 97		3 27	30 47	Left hand dried and wrapped
3 09	29 99	29 98		3 28	30 49	Room 25 1 C
3 10	30 02	30 00		3 29	30 51	
3 11	30 03			3 30	30 54	Hand out of calorimeter at 3 30
3 12	30 05			3 31½		Temp of L is now 29 86 C
3 13	30 08		25 2	3 44½	30 43	Temp of L is now 29 76 C

In James M , a man 60 years old, with a very irregular pulse, enlarged heart and a history of edema of the feet and legs, the flow was 1 99 grams per 100 c c per minute in the right hand, and 1 35 grams in the left (for the last ten minutes in the calorimeters) Anemia was probably not an important factor in the small blood-flow, since the hemoglobin content of the blood was 80 per cent three days before the examination The calculated flows for the last twenty minutes in the calorimeters are 1 56 grams per 100 c c per minute and 1 06 grams for the right and left hands respectively, the ratio between the flows being precisely the same as for the last ten minutes (1 47 1)

James M, a laborer aged 60, was admitted to the City Hospital June 16, 1912 Three weeks before when in another hospital his feet and legs swelled and pitted on pressure On admission to the City Hospital petechiae were present on both legs but there was no edema The right side of the chest lags in inspiration and shows diminished resonance beginning at the sixth interspace in the axillary line, and also posteriorly Fifteen months ago he had dyspneic attacks and the right pleural cavity was tapped June 21, 1912, the right chest was aspirated but no fluid was obtained The left border of heart dulness is outside the nipple line, the right border at the right margin of the sternum The first sound is roughened Pulse irregular, arhythmic, fair volume, sclerosis marked The rectal temperature since his admission has varied from 100 4 to 98 2 F

Examination of the blood flow in the hands was made June 27 1912, on which day he was discharged Hands in bath at 1 34½ p m , in calorimeters at 1 43¾ p m , and out of calorimeters at 2 15

TABLE 2—CALORIMETRIC MEASUREMENTS IN CASE OF JAMES M

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
1 43	31 02	31 12		2 01	30 93	30 97	
1 45	30 99	31 00		2 02	30 93	30 965	
1 46	30 98	31 08	22 9	2 03	30 93	30 955	23 0
1 47	30 98	31 07		2 04	30 925	30 95	
1 48	30 975	31 06	22 9	2 05	30 925	30 945	
1 49	30 97	31 05		2 06	30 93	30 94	23 1
1 50	30 965	31 04	23 0	2 07	30 925	30 935	
1 51	30 96	31 03		2 08	30 925	30 93	
1 52	30 96	31 03		2 09	30 93	30 93	23 1
1 53	30 955	31 025	23 1	2 10	30 925	30 93	
1 54	30 95	31 02		2 11	30 93	30 925	
1 55	30 95	31 015		2 12	30 925	30 925	
1 56	30 94	31 005	23 1	2 13	30 93	30 925	23 0
1 57	30 94	31 00		2 14	30 93	30 92	
1 58	30 94	30 995		2 15	30 925	30 915	
1 59	30 935	30 99		2 27	30 795	30 78	
2 00	30 935	30 98	23 2				

Cooling of calorimeters in twelve minutes, R , 0 13 degrees, L , 0 135 degrees Volume of right hand 368 c c , of left hand 356 c c He is right handed,* wrists very thin, hands skinny Pulse 87 (sitting) Very irregular Rectal temperature 37 65 C Water equivalent of calorimeters with contents, R , 3,389, L , 3,380 Blood pressure, systolic 149 (palpation), 152 (stethoscope), 72 (sound gone) Another observation, systolic 144 (palpation)

A case diagnosed as auricular fibrillation (in N P) is included under this heading, although there was a valvular lesion (mitral stenosis) present as well The case did not come under my observation until the patient had improved considerably under treatment (digitalis), yet even then the flow in the hands was quite small for her age (1 97 grams per 100 c c per minute for the right hand, and 1 59 grams for the left for the last eleven minutes in the calorimeters) The small flow agreed with the persistent coldness of the hands and feet It was not associated with anemia

N P , aged 27, was admitted for her present illness to the City Hospital March 12 1913 (She was first admitted to the hospital April 4, 1910, suffering from rheumatism) Heart Apex beat visible in fifth interspace, one finger's breadth outside the nipple line A presystolic thrill can be felt here Left border two finger's breadth outside the nipple line, right border at the midsternal line upper border of third rib A rough presystolic murmur is heard over a small area at the apex All over the precordia and transmitted to the axilla and back

* [illegible] that a patient was right-handed unless the contrary [illegible]

is heard a soft systolic murmur The pulse shows marked arhythmia Considerable edema The urine, March 13, 1913, contained neither albumin nor casts On May 12, 1913, there was some albumin but no casts Under treatment (including digitalis) the pulse rate diminished The arhythmia became less marked although it did not disappear, and the edema cleared up Her appetite continues poor, but she sleeps well and is not troubled with dyspnea in any position Her hands and feet are always cold She says she is only really warm when surrounded by hot water bottles Polygraph tracings from the jugular and radial taken on May 16, 1913, showed the ventricular form of venous pulse, "a" waves being absent from the jugular tracing The rate of the heart in the semi reclining position was 80 as counted at the apex with the stethoscope, and precisely the same at the wrist The variation in the duration of successive beats is great Twelve successive beats were measured in each of two portions of the tracing In the first series the durations were 1 1, 0 8, 0 9, 0 8, 0 7, 0 65, 0 65, 0 5, 0 6, 0 9, 0 75, 0 9 second, the average being 0 77 second corresponding to 78 beats per minute In the second series the durations were 0 85, 0 65, 0 75, 0 7, 0 7, 0 55, 0 75, 0 85, 0 9, 0 85, 0 95, 0 85 second, the average being 0 78 second corresponding to 77 beats per minute

The blood-flow was examined May 12, 1913 Hands in bath at 2 43 p m, in calorimeters at 2 54½, and out of calorimeters at 3 16 Mouth temperature 36 3 C (the thermometer was in five minutes and special care was taken)

TABLE 3.—CALORIMETRIC MEASUREMENTS IN CASE OF N P

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
2 54	31 56	31 41		3 06	31 455	31 28	23 9
2 55	31 53	31 37		3 07	31 45	31 27	
2 56	31 51	31 36	24 2	3 08	31 45	31 27	
2 57	31 50	31 355		3 09	31 44	31 26	
2 58	31 50	31 35	24 1	3 10	31 43	31 255	23 8
2 59	31 49	31 34		3 11	31 43	31 25	
3 00	31 49	31 33		3 12	31 42	31 24	
3 01	31 48	31 31		3 13	31 42	31 235	
3 02	31 46	31 30		3 14	31 41	31 23	
3 03	31 46	31 29	23 6	3 15	31 41	31 225	
3 04	31 46	31 285		3 16	31 405	31 215	
3 05	31 455	31 28	23 9	3 35	31 19	30 995	

Cooling of calorimeters in 19 minutes, R, 0 215 degrees, L, 0 22 degrees Volume of right hand in calorimeter 298 c c, of left hand 276 c c Pulse (sitting) 75 in one count, 66 in another, irregular and weak It is not easy to count the pulse at the wrist because of the variability in the beat Systolic blood pressure in right arm 95 Difficult to determine the blood-pressure with the stethoscope

A man 44 years old (Jacob S), with mitral stenosis and insufficiency, an irregular heart and weak and irregular pulse, from a study of whose case a diagnosis of auricular fibrillation was made had a flow of only 1 97 grams per 100 c c per minute in the right hand and 0 96 gram in the left, with room temperature 22 2 C The disproportion in the flow in the two hands is associated with old hemiplegia of the left side Immersion of the right hand in warm water caused a great percentile increase in the flow in the left hand (to 1 51 grams per 100 c c per minute for

the first five minutes and 3 86 grams for the next four minutes), although the maximum was still absolutely a small flow. The fair crossed vasomotor reflex from the normal to the formerly paralyzed hand which had largely recovered, contrasts with the absence of reflex in a case of old hemiplegia where there had been no recovery of the hand [8]

Jacob S, a tailor aged 44, height 5 feet, 3½ inches weight 140 pounds, first presented himself at the dispensary Oct 27 1910 Three years before he had rheumatism and had "heart trouble" at that time and also pneumonia Eleven years ago when sitting in a chair he had a left sided paralysis which came on him suddenly He lost consciousness He also had aphasia He could understand what was said but could not speak He recovered his speech in two months The arm and leg recovered slowly There was no disturbance of sensation at the time of the stroke, but two months later he had loss of sensation so that a pin could be stuck into his arm or leg without his knowing it He burnt his left elbow severely without feeling it by leaning on a stove The lines on the left side of the face are not so deep as on the right He drags the left foot somewhat The tongue is protruded in the median line The reflexes are much increased in both upper and lower extremity Babinski's sign can be obtained on the left side Touch and pain are normally appreciated on the left side but heat cannot be distinguished over the left arm The physical signs of chronic bronchitis and emphysema are present Heart slightly enlarged both to right and left Pulmonic second sound accentuated The heart sounds are very irregular both in force and rhythm Pulse rate 80

Nov 5, 1910, a marked presystolic murmur and thrill can be made out

Dec 10, 1910, he is very dyspneic and cannot sleep Ears, fingers, tip of nose and lips are cyanotic Positive venous pulse in the neck The heart dulness extends two fingers outside the nipple line on the left side and two fingers outside the sternal margin on the right There is a sharp first sound preceded by a faint presystolic murmur and followed by a systolic murmur The pulse is small irregular and of very poor volume and tension The left radial pulse is smaller than the right The liver is increased in size to percussion He was received into the hospital to-day where he improved Blood-pressure, systolic 162, diastolic 120 Blood leukocytes, 10,500, hemoglobin, 90 per cent

April 11, 1911, he returned to the dispensary in about the same condition as before he entered the hospital Pulse rapid and irregular

April 25, 1911, enlargement of the heart and arhythmia is still marked Kneepark much exaggerated on the left side When he gets out of breath he feels the left hand getting stiff The difference in size of the two hands is not conspicuous The great difference in the two arms is in the girth of the shoulders the left shoulder being much smaller than the right He cannot completely extend the left forearm on the elbow owing to contracture Sometimes when he is grasping an object in his left hand the fingers remain closed around it involuntarily and he has to release the object with the other hand He lost sexual power at the time of the stroke and has not regained it He has been constipated since the stroke and has had to use cathartics constantly

The blood flow in the hands was measured April 25, 1911 Pulse (sitting) 104 Mouth temperature 36 25 C Hands in bath at 2 31 p m, in calorimeters at 2 42

At 2 55 p m the right hand was put into water at 13 3 C

Calorimeter R was constantly stirred until 3 10 p m After this R and L were only stirred occasionally Cooling of R from 2 55 to 3 10½ is 0 18 degree (in fifteen one-half minutes) Cooling of R from 3 10½ to 3 27 (with little stirring) is 0 15 degree (in sixteen one-half minutes) Cooling of L from 3 10½ to 3 28 (with little stirring) is 0 16 degree (in seventeen one-half min

[8] Post 1911 xii 81

utes) Accordingly with little stirring L cooled at the same rate as R We can therefore assume that with constant stirring this would also be true and that the amount of cooling for L during the observations is 0 18 degree in fifteen and one-half minutes Volume of right hand 390 c c, of left hand 378 c c

TABLE 4.—CALORIMETRIC MEASUREMENTS IN CASE OF JACOB S

Time	Right	Left	Temp Room C	Time	Left	Notes
2 41	29 73	29 72		2 58	29 60	
2 43	29 68	29 65		2 59	29 60	Room 22 7 C
2 44	29 68	29 65	22 0	3 00	29 60	
2 45	29 68	29 64		3 01	29 59	
2 46	29 68	29 64		3 02	29 61	
2 47	29 67	29 63		3 03	29 62	
2 48	29 67	29 63		3 04	29 63	
2 49	29 67	29 62		3 05	29 64	At 3 05 right hand dried and wrapped
2 50	29 68	29 63		3 06	29 64	
2 51	29 68	29 62		3 07	29 65	
2 52	29 68	29 61	22 4	3 08	29 65	
2 53	29 69	29 61		3 09	29 67	
2 54	29 69	29 60		3 10	29 68	At 3 10 hand out of calorimeter
2 55	29 70					
2 56		29 60	22 6	3 10½		Temp of R is now 29 52 C
2 57		29 59		3 27		Temp of R is now 29 37 C
				3 28	29 52	

In a third case diagnosed as one of auricular fibrillation (T F K), a man 57 years old, the flow was 4 45 grams per 100 c c per minute for the right hand, over an eighteen-minute period, and 5 23 grams for the left hand (ratio of the flows in the two hands 1 1 17) For the last nine minutes of this period, the flows were 6 21 grams and 7 06 grams, respectively, for the right and left hands (ratio of the flows in the two hands 1 1 13) These are fair flows for the man's age, indicating that the heart in spite of the irregular action of the ventricle is maintaining a fair output This corresponds entirely with the clinical condition at the time of the blood-flow examination The edema had completely disappeared There was no dyspnea The appetite was good and the patient was no longer confined to his bed Many of the pulse beats at the wrist were of good amplitude, although others were small (Fig 1), while in the case of N P all of the beats were small

T F K, a bricklayer, aged 57, height 5 feet, 9½ inches, weight 140 pounds, was admitted at the City Hospital May 1, 1913 He had typhoid about thirty years ago and rheumatism sixteen or eighteen years ago His present illness began last November His ankles became swollen and also his legs, nearly up to the hips and he lost strength and weight rapidly He noticed at the same time that his pulse was irregular He had pain in the right side of the chest The swelling in the legs had diminished somewhat before he entered the hospital, but on admission edema and cyanosis were still to be observed His chest was barrel shaped with broad costal angle and poor expansion No rales or dulness over the lungs, but there was a friction rub over the anterior surface of the right

lung The cardiac impulse was diffuse, and extended considerably beyond the nipple line on the left side Cardiac dulness enlarged to the left The liver was somewhat enlarged and pulsation evident in the epigastrium The veins in the neck were distended A systolic murmur was heard over the apex His maximum temperature (on May 9) was 103 6 F minimum 97 4 on the same day The maximum temperature on May 16 was 102, May 17, 102, May 19, 101 4 F The pulse rate on May 1 was 120, May 19, 92 The pulse rates were usually above 90 The radial pulse is markedly irregular in amplitude and rhythm, and the irregularity is not affected when respiration is suspended Polygraph tracings from the distended external jugular vein and the radial artery showed that the venous pulse was of the ventricular character (Fig 1) Diagnosis, auricular fibrillation The blood flow in the hands was examined May 19, 1913, at which time the swelling had entirely disappeared from the lower extremities and the patient was allowed to sit up for a portion of the day Hands in bath at 3 14 p m , in calorimeters at 3 26½, and out of calorimeters at 3 47

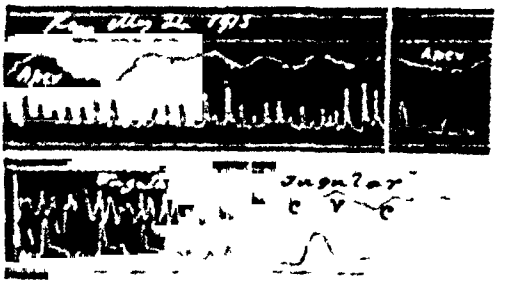


Fig 1—Upper part of tracing, simultaneous record of apex beat and radial pulse The respiratory movements interfered with the apex trace except towards the end of expiration At the right is a portion of the apex tracing which allows a sufficiently good comparison to show that some of the apex beats are only feebly represented on the radial trace The lower portion of the figure gives simultaneous tracings from the external jugular and the radial As in all tracings time is marked in fifths of a second (All tracings reduced to about one half size of originals)

TABLE 5—CALORIMETRIC MEASUREMENTS IN CASE OF T F K

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
3 26	31 59	31 48		3 38	31 63	31 59	24 0
3 28	31 56	31 46	23 5	3 39	31 66	31 625	
3 29	31 565	31 47		3 40	31 695	31 665	23 95
3 30	31 57	31 475		3 41	31 72	31 685	
3 31	1 57	31 48		3 42	31 74	31 73	
3 32	31 57	31 485		3 43	31 78	31 76	24 0
3 33	31 58	31 50	24 2	3 44	31 79	31 77	
34	1 58	31 50		3 45	31 82	31 82	24 1
3 35	31 59	31 525		3 46	31 84	31 86	
3 36	31 61	1 55		3 47	31 88	31 90	
3 37	1 62	31 56		4 16	31 52	31 555	

Rectal temperature 37 35 degrees Blood pressure in left brachial, maximum systolic (when the larger pulsations begin to be heard) 111, 58 (sound gone) Another observation, maximum systolic 114, 58 (sound gone)

In two cases in which acute symptoms diagnosed as due to myocarditis had been present, but which had markedly improved under treatment so that the patients were able to come without trouble to the dispensary, a fair hand flow was found Both were men 59 years old In one of these patients (J S W) who had been in the hospital two years before and had been near death, the flow was 6 82 grams per 100 c c per minute in the right hand and 6 58 grams in the left, with room temperature at 21 9 C Simultaneous tracings from the jugular bulb, the radial artery and the apex showed that the *"c"* wave was notably large, the *a* wave was also well marked All the ventricular beats were recognizable on the radial trace

J S W, a fireman, colored, height 5 feet, 10 inches, weight 160 pounds, born in 1851, was first admitted to the dispensary Feb 21, 1905, complaining of pain in chest and cough The heart sounds were faint but clear No murmurs Heart dulness enlarged to the left well beyond the mammary line The pulse was irregular A diagnosis of myocarditis was made April 13, 1908, he was again admitted to the dispensary Heart enlarged to the left two fingers breadth beyond the mammary line Pulse rapid and irregular Some albumin in the urine Nov 4 1908, he was admitted to Lakeside Hospital and again on Feb

TABLE 6.—CALORIMETRIC MEASUREMENTS IN CASE OF J S W

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
3 10	29 40	29 29		3 22	29 96	29 86	
3 14	29 47	29 38		3 23	30 00	29 91	
3 15	29 53	29 43		3 24	30 08	29 97	
3 16	29 59	29 50		3 26	30 20	30 10	
3 17	29 66	29 55		3 27	30 23	30 12	
3 18	29 73	29 61		3 34	30 16	30 06	
3 19	29 78	29 67		3 39	30 09	30 00	
3 20	29 83	29 71		3 51	29 96	29 88	
3 21	29 89	29 80					

20, 1909 He had attacks of syncope with fluttering in the chest and came near death The pulse-rate was 120, systolic blood-pressure 160 Pulse regular and all the beats reached the wrist Under treatment the pulse rate diminished and as it slowed the arhythmia returned (premature contractions) March 19, 1909, the rate was 68, systolic blood pressure 160 Tracings from the bulbus venosus showed well marked *a*, *c* and *v* waves of which the *c* wave was very large Presystolic systolic and diastolic pulsations were visible over the jugular bulb He was discharged from the hospital May 5, 1909, improved Oct 5, 1909, he reported at the dispensary that he had gained 20 pounds since leaving the hospital Oct 4, 1910, he was feeling better, but had occasional smothering sensations There was now no arhythmia and no albumin in the urine

The flow in the hands was measured Jan 10, 1911 He feels much improved Systolic pressure still very high, when last taken 210 Hands in bath at 3 00 p m , in calorimeters at 3 11½, and out of calorimeters at 3 27, 3,050 c c of water in each calorimeter Room temperature 21 7 C (Table 6)

Volume of right hand 570 c.c., of left hand 570 c.c. His hands are very large. Pulse 60 (sitting). Mouth temperature 36.5° C. Rectal temperature 37.6° C. Room temperature at end of observations 22.2° C.

Since this time he has continued to report at the dispensary and has been more than once in the hospital with the signs of cardiac breakdown. On Dec. 7, 1912, "the left border of the cardiac dulness is at the anterior axillary line, and the right one finger's breadth beyond the right sternal border. Heart thumping and somewhat irregular. Feb. 11, 1913, spitting blood and short of breath. Right border of cardiac dulness two fingers breadth to right of sternal margin. Sounds distant and irregular. No murmurs. Rales throughout both lungs." In June, 1913, eight years from the beginning of his illness, he was still coming to the dispensary and had periods of temporary improvement.

The other patient (Peter B.) was twice examined. At the first examination he had a flow of 6.11 grams per 100 c.c. of hand per minute in the right hand and 5.99 grams in the left, with room temperature 21.5° C. This is by no means a large flow for the temperature, and while fair reflex vasodilatation was obtained by the application of warmth to the contralateral hand, cold did not cause vasoconstriction sufficient to reduce the flow to the initial amount. This agrees well with the fact that arteriosclerosis was distinct for reflex vasoconstriction is not well obtained in this condition. Almost a month later his hand flow was again measured. He had meanwhile much improved. The flow was 9.95 grams per 100 c.c. per minute for the right and 7.46 grams for the left hand. However, the room temperature was relatively high (26.6° C.) and this probably accounted for a portion of the increase in the flow. The vasodilatation consequent on the high external temperature was considerable and permitted a small reflex vasoconstriction when the contralateral hand was immersed in cold water.

Peter B., a janitor aged 59, height 5 feet, 2 inches, weight 126 pounds, was under observation at the dispensary with symptoms diagnosed as due to myocarditis. For many years he did heavy work in a gas works. Though his work is now light he has had to give it up for the present. He suffered greatly from rheumatism till about fifteen years ago, since which time he has not been troubled. His joints would swell and he could not walk. He is married and has seven children all in good health. His present illness dates from somewhat less than two years ago when he suddenly became unconscious while mowing the lawn. He remained unconscious for five minutes. [illegible]

The flow in the hands was first examined April 21, 1911 Pulse (sitting) 80 Mouth temperature 36 45 C Room temperature 24 8 C Hands in bath at 2 31½ p m, in calorimeters at 2 42 At 2 53 left hand put into water at 42 9 C

TABLE 7.—CALORIMETRIC MEASUREMENTS IN CASE OF PETER B

Time	Right	Left	Room Temp C	Time	Right	Notes
2 40½	30 04	30 03		3 00	30 57	
2 43	30 01	30 02		3 01	30 60	
2 44	30 03	30 03	24 7	3 02	30 63	
2 45	30 06	30 06		3 03	30 69	At 3 03 put left hand in water at 8 C
2 46	30 09	30 09		3 04	30 71	Room 24 3 C
2 47	30 11	30 10		3 05	30 77	
2 48	30 14	30 12		3 06	30 79	
2 49	30 17	30 14		3 07	30 81	
2 50	30 20	30 18		3 08	30 83	
2 51	30 24	30 21	24 3	3 09	30 87	
2 52	30 28	30 23		3 10	30 89	
2 53	30 30	30 25		3 11	30 91	At 3 11 dried left hand and exposed it for inspection Then wrapped it in warm cloth
2 54	30 32			3 12	30 93	
2 55	30 36		24 4	3 13	30 97	
2 56	30 40			3 14	31 00	Room 24 2 C
2 57	30 44			3 15	31 04	Hand out of calorimeter at 3 15
2 58	30 49		24 2	3 30	30 89	At 3 30 temp L 29 95 C
2 59	30 52					

Cooling of calorimeters, R, 0 15 degree in fifteen minutes, L, 0 30 degree in thirty-seven minutes Volume of right hand 360 c c, of left 320 c c Water equivalent of calorimeters with contents, R, 3,383, L, 3,351

Second examination May 17 1911 He is feeling much better than when last examined He was being treated with potassium iodid and nitroglycerin Although he has had slight attacks of dizziness and "feeling stupid," he has not fallen or "gone stiff" There is no arhythmia now, nor can any murmurs be detected Pressure on the vagus in the neck readily inhibits the heart causing the pulse at the wrist to disappear for a beat or two He feels dizzy during the compression, and he can be made unconscious at any time by pressing on the vagus

Hands in bath at 2 33 p m, in calorimeters at 2 43½, mouth temperature 36 5 C, pulse (sitting) 84 At 2 55 p m left hand put into water at 8 C

Cooling of calorimeters, R, 0 07 degree in ten minutes, L, 0 17 degree in twenty-nine minutes Volume of right hand in calorimeter 410 c c, of left hand 394 c c The marks were put higher up than usual as his hands were short The lower mark was about the middle instead of the lower border of the styloid process of the ulna He seemed gradually to improve and reported at his last visit to the dispensary June 7, 1911, that he was better Pulse 84 (See Table 8)

II VALVULAR LESIONS

The flow in these cases may be normal or much less than normal depending on the degree of compensation Since anemia may be associated with a valvular lesion, it may be a factor in diminishing the hand

flow in the way already discussed in a previous paper.[7] Since, further, the degree of involvement of the myocardium will differ in different cases with one and the same valvular lesion, it is not to be expected that a relatively large flow should always be associated with one particular type of valvular defect and a small flow with another. Nevertheless, so far as can be judged from the series of cases examined, certain of the valve lesions are more apt to be associated with the normal flow than others or even with a flow somewhat above the normal. Mitral insufficiency, for instance, it would seem, is less likely to be associated with a diminished

TABLE 8.—CALORIMETRIC MEASUREMENTS IN CASE OF PETER B. SECOND DAY.

Time	Right	Left	Room Temp. C.	Time	Right	Notes
2 43	30 99	30 18		3 00	31 125	
2 45	30 25	30 09	26 4	3 01	31 16	
2 46	30 42	30 27		3 02	31 195	
2 47	30 51	30 39		3 03	31 23	
2 48	30 57	30 38	26 5	3 04	31 29	
2 49	30 63	30 495		3 05	31 325	At 3 05 left hand put in water at 43 C.
2 50	30 68	30 455		3 06	31 355	
2 51	30 73	30 50		3 07	31 38	
2 52	30 79	30 535		3 08	31 41	
2 53	30 835	30 57		3 09	31 44	
2 54	30 88	30 60		3 10	31 475	Room 26 7 C.
2 55	30 915	30 625		3 11	31 505	
2 56	30 94			3 12	31 53	
2 57	30 99		26 8	3 13	31 565	
2 58	31 035			3 14	31 59	Room 26 6 C. Hand out of calorimeter at 3 14
2 59	31 08			3 24	31 52	At 3 24 temp L 30 455 C

hand flow than aortic insufficiency. In the only case in which a diagnosis of pure aortic stenosis seemed justified, the hand flow was normal, and this accord with the clinical condition of the patient, who indeed did not enter the hospital for his heart lesion, but for a surgical cause. To emphasize the fact, however, that the mere naming of the valvular lesion is no indication of the amount of the hand flow, we shall begin the discussion of the group of cases with one of mitral incompetence in which the flow is very markedly diminished.

was treated with digitalis April 9, 1912, the blood flow in the hands was examined Hands in bath at 2 21 p m, in calorimeters at 2 31 He had some cough during the observations Systolic blood pressure 122 Pulse 70 At 2 47 p m left hand put into water at 42 7 C

TABLE 9.—CALORIMETRIC MEASUREMENTS IN CASE OF JOHN T

Time	Right	Left	Room Temp C	Time	Right	Notes
2 30½	31 40	31 49		2 48	31 385	
2 32	31 35	31 44	24 3	2 49	31 385	
2 33	31 35	31 44		2 50	31 39	Room 24 3 C
2 34	31 34	31 43		2 51	31 39	
2 35	31 34	31 43	24 1	2 52	31 395	
2 36	31 335	31 425		2 53	31 395	
2 37	31 335	31 425		2 54	31 395	
2 38	31 34	31 43		2 55	31 40	Hand out of calorimeter Room 24 3 C
2 39	31 345	31 435		3 11	31 23	At 3 11 temp L 31 225 C
2 40	31 35	31 435				
2 41	31 35	31 435	24 3			
2 42	31 36	31 435				
2 43	31 365	31 44				
2 44	31 37	31 445				
2 45	31 37	31 45				
2 46	31 38	31 455	24 6			
2 47	31 38	31 455				

Cooling of calorimeters, R, 0 17 degree in sixteen minutes, L, 0 23 degree in twenty-four minutes Volume of right hand 450 c c, of left hand 438 c c Water equivalent of calorimeters with contents, R, 3,455, L, 3,445 Rectal temperature 37 95 C

April 24, 1912, he was discharged "improved"

In this case the flow was only 2 09 grams per 100 c c of hand per minute in the right hand, and 1 67 grams in the left, with room temperature 24 4 C, a flow very much too small for his age

In a case of mitral stenosis, mitral and tricuspid insufficiency in a man 63 years old (James H), the flow, measured on an exceedingly hot day with a room temperature of nearly 31 C, was 7 31 grams per 100 c c of hand per minute for the right hand and 6 41 grams for the left Probably not more than one-third to one-half of the flow to be expected in a healthy young adult for that room temperature Measurements made at such high room temperatures do not lend themselves readily to comparisons The examination was made on this day purposely to observe the effect of maximal vasodilatation on the flow in this case, since at two previous examinations the hand flow seemed to be greatly diminished by persistent local vasoconstriction which the patient attributed to the low temperature of the wards He said he had felt chilly all the time, but to-day he felt warm The pulse showed arhythmia (Fig 2), partly respiratory

James H a butler aged 63 admitted to the City Hospital May 2, 1913 suffering from chronic endocarditis with mitral stenosis and insufficiency and tricuspid insufficiency No history of rheumatism He complains of weakness and shortness of breath for the last three or four weeks His feet swell at times Ears lips, nails and mucous membranes were markedly cyanotic on admission There was slight edema of the legs with great varicosity of the leg veins Marked

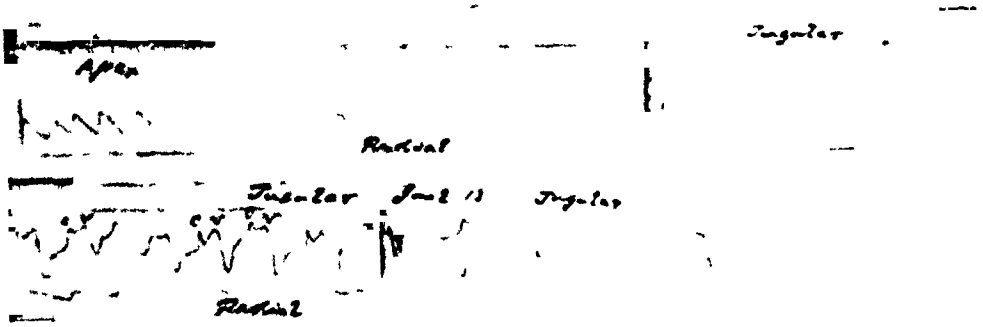


Fig 2—Tracings from James H

engorgement and pulsation of the veins of the neck Lungs revealed nothing special on physical examination Heart enlarged Left border of cardiac dulness at the anterior axillary line in the sixth interspace, right border 3 cm to right of sternum A presystolic thrill followed by impact and systolic thrill palpable over apex Liver pulsatile and the lower edge palpable 8 cm below the costal margin Radial pulse markedly irregular and arhythmic with fair volume and tension, fairly well sustained He was discharged May 17 improved He went to work but became ill again and returned to the hospital May 23, 1913

TABLE 10—CALORIMETRIC MEASUREMENTS IN CASE OF JAMES H

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
2 00	31 30	31 21		2 17	31 07	30 98	23 3
2 02	31 25	31 17	23 1	2 18	31 055	30 97	
2 03	31 23	31 16		2 19	31 01	30 96	
2 04	31 22	31 15		2 20	31 03	30 955	23 4
2 05	31 20	31 13	23 3	2 21	31 02	30 95	
2 06	31 19	31 12		2 22	31 01	30 945	
2 07	31 18	31 105	23 3	2 23	31 00	30 94	23 4
2 08	31 17	31 09		2 24	30 995	30 93	
2 09	31 15	31 08	23 2	2 25	30 995	30 925	
2 10	31 13	31 06		2 26	30 99	30 915	23 5
2 11	31 125	31 055		2 27	30 98	30 90	
2 12	31 11	31 05	23 2	2 28	30 975	30 895	
2 13	31 10	31 04		2 29	30 97	30 89	
2 14	31 09	31 03	23 2	2 30	30 96	30 88	23 6
2 15	31 08	31 01		3 21	30 395	30 34	
2 16	31 07	30 99					

Cooling of calorimeters in fifty one minutes, R, 0 565 degree, L, 0 54 degree Volume of right hand 418 c c, of left hand 396 c c The hands do not feel cold to the touch on coming out of the calorimeters They are fairly red The veins on the back of the hand and in the forearm are large, but fill with marked slowness on being emptied Blood-pressure, left arm, systolic 136, 63 (sound gone) Another observation 134, 68 There was no sensible interval between the change in sound and its disappearance A few of the larger beats were heard a little before the readings given as systolic pressure The number of waves coming through increased as the pressure fell

The flow in the hands in this examination works out at only 0 80 gram per 100 c c per minute for the right hand, and 0 64 gram for the left, for the last ten minutes of immersion in the calorimeters If the whole period in the calorimeters is taken, the calculated flow is still less, as is always the case with a very small flow, the period required for the attainment of the steady state where the thermometers are changing uniformly being long

A second examination on June 2, 1913, gave an even smaller calculated flow (0 40 gram per 100 c c per minute) for the last ten minutes in the calorimeters The temperature readings followed precisely the same course as at the first examination The question arises how such small flows can be reconciled with the fairly good volume of the arterial pulse at the wrist The explanation is found partly in the fact that the venous return was hindered as indicated by the appearance of the veins The increase in venous pressure while it did not hinder the conduction of the arterial pulse wave, apparently diminished the rate of translation of the blood A second factor in the small flow was apparently a persistent local vasoconstriction in the hands That this second factor was concerned is shown by the great improvement in the hand flow at the third examination, although the pulse-rate, far from being accelerated, was even slower than at the previous examinations

Third examination June 16, 1913 He is feeling well, only weak No dyspnea and no cough whatever The day is very warm and the room temperature the highest in the whole series of observations The veins on his hands and arms fill much more quickly after being emptied than on the two previous occasions Amplitude of the pulse at the wrist is fair, but not strikingly greater than at the other examinations Pulse 57 (sitting), with some irregularity but not so marked as before Hands in bath at 4 06½ p m, in calorimeters at 4 15½, and out of calorimeters at 4 27

Cooling of calorimeters in ten minutes, R, 0 045 degree, L, 0 04 degree Volume of right hand in calorimeter 408 c c, of left hand 392 c c Water equivalent of calorimeters with contents, R, 3,421, L, 3,409 Blood-pressure in left brachial, systolic 110, another observation 106, 80 (change in sound) Rectal temperature 37 6 C (See Table 11)

The general cutaneous vasodilatation at the third examination was evident and is doubtless related to the decidedly diminished systolic pressure The hands shared in this general vasodilatation, and it is

probable for this reason that the hand flow gives an index to the heart output Since the hand flow with practically maximal vasodilatation is not half that of a young adult at this room temperature, the heart output is probably at most 50 per cent of that of a healthy young adult The three observations together show that in this man of 63 the range of the vasomotor adaptation of the hand blood-vessels is still considerable, a confirmation of his statement that the hot weather suited him

TABLE 11 —Calorimetric Measurements in Third Examination of James H

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
4 15	31 45	31 50		4 22	31 71	31 72	30 9
4 16	31 46	31 52		4 23	31 765	31 76	
4 17	31 49	31 54		4 24	31 795	31 83	
4 18	31 54	31 58	30 8	4 25	31 86	31 84	30 9
4 19	31 58	31 62		4 26	31 89	31 87	
4 20	31 62	31 655	30 9	4 27	31 925	31 90	
4 21	31 67	31 69		4 37	31 88	31 86	

From the standpoint of the technic of the method it ought still to be mentioned that a local vasoconstriction so persistent as not to be overcome during the usual period of an examination (the disappearance of it being shown by a rather abrupt and henceforth steady rise of the thermometers) is rare in hospital cases, although in hands long exposed to cold it may be frequently met with Where it exists the results cannot, of course, be used as average flows for the patient in question, although the intensity and persistence of the vasoconstriction may be of interest in relation to the patient's condition

In a case of badly broken compensation in a boy (Joseph Z), 16 years old, suffering from chronic endocarditis with aortic insufficiency and stenosis and mitral insufficiency, the flow in the right hand was 6 28 grams per 100 c c per minute, and in the left hand 4 79 grams This is much too small for the age of the patient and the very high room temperature (28 1 C) He was obviously in bad condition Immersion of the left hand in cold water was followed by but a small diminution of the flow in the right, and immersion of the left hand in warm water by no increase at all, either because the dyspneic condition of the blood caused increased stimulation of the vasoconstrictors of the hand which the vasodilator effect of warmth to the contralateral hand could not overcome, or because the vasoconstrictor tone was already much diminished under the influence of the warm room, although, owing to the handicap of the heart, this was not shown in a large flow Another possibility is that the dyspneic condition of the blood may have rendered the vasomotor centers less excitable to this form of afferent stimulation

Joseph Z, boy aged 16, was admitted to the dispensary Nov 11, 1910, suffering from aortic insufficiency and stenosis with attacks of acute pulmonary edema He has had rheumatism for ten years He was in the hospital three weeks for heart trouble which started four years ago Has dyspnea and weakness His heart is enlarged 16 cm to the left, and 5 cm to the right of the median line Pulsation in the arteries of the neck Apex beat heaving Systolic impulse over apex A systolic thrill can be felt to the right of the sternum A double murmur over the apex and louder over the aortic region Pulse 95 Moderate tension, rather slow rise and quick fall Pistol shot in femorals Capillary pulse

Nov 15, 1910, he was admitted to the hospital where he improved and after his discharge he remained well, keeping in bed, however, most of the time

Feb 2, 1911 When in the dispensary he had an attack of palpitation during which the pulse rate rose to 160, with great throbbing in the carotids and general cyanosis He began to spit up pinkish frothy liquid The attack lasted forty-five minutes He was again admitted to the house, where the diagnosis of chronic endocarditis, mitral insufficiency and aortic insufficiency, was made He suffered from occasional attacks of tachycardia and shortness of breath The blood-examination showed hemoglobin 90 per cent He was discharged July 5, 1911, "improved"

July 14, 1911, he had an attack in the dispensary There was great pain referred to the right infrascapular region He received morphin (grain ⅛) and atropin sulphate (gr 1/300) hypodermically to relieve and calm him, and fifteen minutes later the blood flow in his hands was measured Pulse (sitting) 140 Hands in bath at 3 01 p m, in calorimeters at 3 11½ Mouth temperature 38 15 C At 3 21 p m left hand was put into water at 8 C

TABLE 12.—CALORIMETRIC MEASUREMENTS IN CASE OF JOSEPH Z

Time	Right	Left	Room Temp C	Time	Right	Notes
3 11	29 87	29 905		3 27	30 59	
3 12	29 88	29 915		3 28	30 60	Pulse 132
3 13	29 91	29 93		3 29	30 635	
3 14	29 98	29 97		3 30	30 695	At 3 30 put left hand in water at 43 C
3 15	30 07	30 04	28 1	3 31	30 735	
3 16	30 13	30 10		3 32	30 775	Room 28 4 C
3 17	30 21	30 13		3 33	30 81	
3 18	30 22	30 15		3 34	30 87	
3 19	30 27	30 20		3 35	30 895	
3 20	30 29	30 22		3 36	30 93	
3 21	30 32	30 235		3 37	30 975	Room 28 3 C
3 22	30 34			3 38	31 01	3 38 hand out calorimeter
3 23	30 37	30 22		3 38½		Temp of L 30 16 C
3 24	30 40			3 48	30 97	Pulse 120 Mouth temp
3 25	30 49		28 1			38 45 C
3 26	30 54					

Cooling of calorimeters, R, 0 04 C in ten minutes, L, 0 06 C in fifteen one half minutes Volume of right hand 406 c c, of left 412 c c He is right handed Water equivalent of calorimeters with contents, R, 3,420, L, 3 425

He attended from time to time at the dispensary feeling pretty well for awhile, but compensation broke down again and on March 3, 1913, he had again to seek admission to Lakeside Hospital with the cardiac dulness enlarged to the left to the mid axillary line

In another case of aortic stenosis and insufficiency and mitral insufficiency with fair compensation at the time of the blood-flow examination in a man (Matthew K), 42 years old, the flow was 7 20 grams per 100 c c per minute in the right, and 5 49 grams in the left, with room temperature 22 5 C, a flow somewhat below the normal for his age and the external temperature, although by no means remarkably small He had no edema and no dyspnea, but he complained of pain over the heart and was getting weaker

Mat K, aged 42, height 5 feet, 7 inches, weight 165 pounds, a worker in a railway shop, was admitted at the dispensary Dec 2, 1910, suffering from aortic stenosis and insufficiency and mitral insufficiency His illness began two months before and he has been getting worse ever since He feels chilly at times His weakness is increasing each day and he has had to give up work He suffers from pain over the heart, palpitation and inability to sleep There is a probable history of rheumatic fever seven years ago The left border of the cardiac dulness is 12½ cm to the left of the mid-sternal line, the right border 4 cm to the right of it, upper border at third rib No capillary pulse, no edema, urine normal

The blood-flow in the hands was measured Jan 24, 1911 He has no shortness of breath The systolic blood pressure is 170 Mouth temperature 36 6 C, pulse (sitting) 80 Hands in bath at 3 16 p m, in calorimeters at 3 27, out of calorimeters at 3 42 There was 3,050 c c of water in each calorimeter Room temperature 22 0 C before the observations were begun

TABLE 13—Calorimeter Measurements in Case of Mat K

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
3 26	29 42	29 19		3 37	29 73	29 43	
3 28	29 40	29 18		3 38	29 78	29 46	
3 29	29 42	29 18		3 39	29 82	29 48	
3 30	29 44	29 20		3 40	29 86	29 51	
3 31	29 47	29 21		3 41	29 90	29 54	
3 33	29 54	29 29		3 42	29 96	29 58	23 1
3 34	29 60	29 33		3 59	29 76	29 42	
3 35	29 64	29 37		4 04	29 70	29 37	
3 36	29 70	29 40					

Cooling of calorimeters in twenty-two minutes, R, 0 26 degree, L, 0 21 degree Volume of right hand in calorimeter 440 c c, of left 435 c c He is right handed

He continued to come to the dispensary On Feb 2, 1911, he complained of pain over the precordia and down the shoulder and arm His last visit was on April 2, 1913, when his condition was not essentially altered

In a case of mitral and tricuspid insufficiency (P B) with venous engorgement, edema of the ankles and fluid in the peritoneal cavity, a perfectly good flow was seen in the hands (12 80 grams per 100 c c per minute for the right and 13 46 grams for the left, with room temperature 25 5 C) A fair reflex vasoconstriction was obtained by the application of cold to the contralateral hand, but the application of warmth failed to increase the flow even to the initial level, owing no doubt to the fact

that the vessels were already so much dilated There was no dyspnea during the experiment, and the driving power of the heart with its obviously powerful stroke was adequate to maintain a normal flow through the periphery The vasomotor centers seem to have retained their normal excitability The cord, therefore, in spite of the venous engorgement, was not suffering appreciably at the time

P B, section hand on a railway aged 31, height 5 feet, 11 inches, was admitted to the dispensary May 12, 1911, with mitral and tricuspid insufficiency He has been ill for eighteen or nineteen months, during which time he has been unable to work He had acute articular rheumatism nineteen months before admission, had swollen feet, enlargement of the abdomen and palpitation of the heart and was in bed six weeks The left border of the cardiac dulness is at the mid-axillary line There is a large diffuse area of cardiac pulsation visible (positive systolic) and the framework of the chest undulates with each beat Positive venous pulsation in both jugulars Liver much enlarged with marked systolic pulsation There is venous engorgement, edema of the ankles and fluid in the peritoneal cavity

The blood-flow in the hands was measured May 12, 1912 There was no dyspnea during the examination Pulse (sitting) 100 Mouth temperature 36 9 C Hands put into bath at 2 29 p m, into calorimeters at 2 39 At 2 50 p m left hand put into water at 8 C

TABLE 14.—CALORIMETRIC MEASUREMENTS IN CASE OF P B

Time	Right	Left	Room Temp C	Time	Right	Notes
2 38	30 21	30 11		2 57	31 67	
2 40	30 28	30 19		2 58	31 71	
2 41	30 375	30 28		2 59	31 77	
2 42	30 48	30 40		3 00	31 82	Room 25 7 C
2 43	30 59	30 495		3 01	31 875	At 3 01 put left hand in water at 43 1 C
2 44	30 68	30 61	25 5	3 02	31 91	
2 45	30 79	30 71		3 03	31 97	
2 46	30 88	30 80		3 04	32 02	Room 25 5 C
2 47	30 97	30 875		3 05	32 085	
2 48	31 06	30 96		3 06	32 135	
2 49	31 14	31 06	25 6	3 07	32 195	
2 50	31 23	31 16		3 08	32 26	
2 51	31 295			3 09	32 32	
2 52	31 345			3 10	32 38	3 10 hand out calorimeter
2 53	31 395			3 10½		Temp of L is now 30 98 Room 25 7 C
2 54	31 475			3 22½	32 25	
2 55	31 545		25 8	3 23½		Temp L is now 30 88 C
2 56	31 605					

Cooling of calorimeters, R, 0 13 degree in twelve one half minutes, L, 0 28 degree in thirty two minutes Volume of right hand 510 c c, of left hand 480 c c

He entered Lakeside Hospital August 21, 1911 He had then neither cyanosis nor edema, but there was still fluid in the abdomen The apparent incongruity between the distention of the abdomen, the pulsation of the liver and the great comfort of the patient was remarked Heart dulness extends on the right to 7 cm

from the mid-sternal line in the third interspace, on the left to the anterior axillary line in the sixth interspace The blood examination showed 90 per cent hemoglobin He again entered Lakeside Hospital Nov 2, 1911, and was discharged "improved" Feb 20, 1912

The large flow in P B contrasts with the small flow in Joseph Z, in whom the delivery of blood to the systemic vessels was seriously interfered with by the insufficiency of the aortic valve

In Ernest M, another case of mitral insufficiency with chronic bronchitis, in a boy 14 years old, the flow was also good (12 74 grams per 100 c c per minute for the right hand and 12 45 grams for the left hand, with room temperature 23 4 C) Compensation was so far established that he was able to come to the dispensary and had no dyspnea during the examination

Ernest M, boy, aged 14, height 5 feet, 1¼ inches, was admitted at the dispensary Jan 26, 1911, suffering from mitral insufficiency with chronic bronchitis The fingers of both hands show well marked clubbing He has had a cough ever since he was two years old He still has the cough and is getting thinner His arms are thin for his age He gives a history of heart trouble He has never been very strong and is not well enough to go to school His appetite is good Up to the present time there has never been any blood in the sputum, but on Feb 7, 1911, he stated that since his last visit he spat some blood The sputum examination was always negative as regards tubercle bacilli

The hand flow was measured Jan 26, 1911 Mouth temperature 38 2 C Rectal temperature 38 5 C Hands in bath at 2 56 p m , in calorimeters at 3 07, and out of calorimeters at 3 23 There was 3,150 c c of water in each calorimeter Pulse (sitting) 116 The weather was mild with rain Room 23 4 C

TABLE 15—Calorimetric Measurements in Case of Ernest M

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
3 06	29 71	29 68		3 17	30 18	30 10	
3 09	29 73	29 68		3 18	30 24	30 16	23 4
3 10	29 77	29 72		3 20	30 34	30 27	
3 12	29 89	29 82		3 21	30 40	30 33	
3 13	29 96	29 89		3 22	30 45	30 39	
3 14	30 00	29 93		3 23	30 50	30 42	23 1
3 15	30 06	29 99		3 42	30 30	30 27	22 7
3 16	30 12	30 05					

Cooling of calorimeters in nineteen minutes, R, 0 20 degree, L, 0 15 degree L was on the side of the room next to the radiator, though at a good distance from it A small centrifuge had been started at 3 23 at the side of the room nearest to R The difference in the rate of cooling of the calorimeters, which is decidedly greater than usual, may be thus accounted for Volume of right hand 255 c c, of left 240 c c

In another boy (Frank C), 17 years old, with mitral stenosis and insufficiency, the flow was also quite normal in amount (11 25 grams for the right and 11 58 grams for the left hand, with room temperature 22 4

C) Compensation was fairly established The protocol has already been published[6] Later he was admitted several times to Lakeside Hospital suffering from broken compensation and was discharged for the last time on March 4, 1912 He afterward died

One of the most interesting cases was that of Albert H, since the heart underwent rapid deterioration within the period of the observations and the patient died soon after the last examination, when an autopsy was obtained The patient was a man 31 years of age with a clinical diagnosis of mitral and tricuspid insufficiency, confirmed by the autopsy, which in addition showed pericarditis with large effusion into the pericardial sac There was no doubt that the myocardium had suffered The patient suffered much from dyspnea if he attempted to lie down The flow in the right hand was 4 08 grams per 100 c c per minute, and in the left 4 54 grams, with room temperature 24 7 C, a distinctly subnormal flow Reflex vasoconstriction of the hand was easy to obtain on immersion of the contralateral hand in cold water, but no reflex vasodilatation could be obtained, which agreed well with the obviously poor filling of the vessels of the hand Ten days later he was again examined The abdomen was now full of liquid, the legs and feet greatly swollen, the heart-sounds weak and the pulse at the wrists so weak that the heart-rate was counted with the stethoscope The hands also had increased in size The blood-flow in the hands was very small and what was considered significant at the time, less than half its amount at the last examination (2 0 grams for the right and 1 88 grams for the left) This was for a period of seven minutes, beginning fifteen minutes after insertion of the hands in the calorimeters For the first fifteen minutes the flow was still less The patient said he felt better, but the great decline in the blood-flow was remarked as an ominous symptom He died in the hospital ten days after the last examination

Albert H, colored hotel porter, aged 31, was admitted to the City Hospital April 3, 1912, complaining of dyspnea, cough and swelling of the extremities He had rheumatism three weeks before admission and the legs were very painful The edema and dyspnea began one week before admission He has grown worse, coughs most of the time and is very tired on any exertion The physical signs of fluid in the right pleural cavity are present Heart dulness, upper border third rib, right border parasternal line, left border midway between nipple and anterior axillary line The rate of the heart is rapid, the sounds feeble There is a soft mitral systolic murmur with reduplication of the second sound Radial pulse small and easily compressible No fibrosis Great edema of scrotum and prepuce, and edema of lower extremities Albumin in urine April 5 and April 7 the dyspnea and edema were less, the mitral murmur not so pronounced, the pulse soft and not well sustained April 9, on which day the hand flow was first measured, the signs of an increase in the fluid in the right pleural cavity were present The heart boundaries were the same as before and the edema was still considerable Seven hundred and fifty c c of liquid was aspirated from the right thorax before the blood flow examination His maximum temperature on April 3 was 100 6 F It rose gradually till April 9 when it reached 104 5 F The systolic blood pressure on April 5 was 152, April 7, 160, April 9, 160

Hands in bath at 1 09 p m, in calorimeters at 1 19 Mouth temperature 37 8 C At 1 31 p m left hand put into water at 7 9 C

TABLE 16—Calorimetric Measurements in Case of Albert H

Time	Right	Left	Room Temp C	Time	Right	Notes
1 18	30 82	30 78		1 35	31 13	
1 20	30 81	30 75		1 36	31 14	
1 21	30 84	30 76	24 6	1 37	31 16	Room 24 7 C
1 22	30 87	30 79		1 39	31 19	
1 23	30 89	30 82		1 40	31 195	At 1 40 left hand put in water at 43 C
1 24	30 90	30 85	24 9	1 41	31 21	
1 25	30 96	30 90		1 42	31 22	Room 24 8 C
1 26	31 03	30 96		1 43	31 23	
1 27	31 07	31 00		1 44	31 245	
1 28	31 08	31 02	24 6	1 45	31 26	
1 29	31 085	31 04		1 46	31 28	
1 30	31 095	31 07		1 47	31 295	
1 31	31 09	31 09		1 48	31 305	
1 32	31 10			1 49	31 325	
1 33	31 105		24 5	1 50	31 33	1 50 hand out calorimeter
1 34	31 115			1 59	31 23	Temp of L is 30 81 C at 1 59

Cooling of calorimeters, R, 0 10 degree in nine minutes, L, 0 28 degree in twenty-eight minutes Volume of right hand 447 c c, of left hand 450 c c He says he does most of his work left handed Water equivalent of calorimeters with contents, R, 3,452, L, 3,455 Pulse (lying down) 120 Rectal temperature 39 1 C

April 10 his maximum temperature was 103 8 F, April 14, 103 3 F April 17, "the dulness in the right thorax begins as high as the fifth rib posteriorly Heart boundary is the same" Systolic blood-pressure April 16, 130, April 18, 140, April 19, 130

Second examination of the hand flow, April 19, 1912 The abdomen now contains much liquid The legs and feet are greatly swollen and he cannot sleep because he is unable to lie down on account of dyspnea The heart sounds are weak and the pulse at the wrist barely discernible He says he feels better than before the tapping on April 9, but he seems worse than when examined after the tapping on that date The arms and hands, according to his statement, are not swollen, but simply fat and there is no pitting Yet measurement of the volume of the hands demonstrates an increase in their size, although the edema is not great enough to be detected by pitting He has no fever now

Hands in bath at 1 58 p m, in calorimeters at 2 09 p m, and out of calorimeters at 2 45 At 2 35 the right hand was put into water at 42 7 C

Cooling of calorimeters, R, 0 295 degree in twenty-eight and one-half minutes L, 0 17 degree in eighteen and one half minutes Pulse 102 (counted with stethoscope) Rectal temperature 37 75 C It was noticed that he now has piles He never had them before Volume of right hand in calorimeter 475 c c, of left hand 505 c c Water equivalent of calorimeters with contents, R, 3 475, L, 3,499 (See Table 17)

April 26, 1912, edema was general in the legs, arms and face Maximum temperature 102 4 F He died April 29 Results of necropsy Pericarditis, 600 c c fluid in the pericardial sac Mitral valve, edges thickened, insufficient Tricuspid valve, insufficient One thousand c c fluid in the abdomen Chronic passive congestion of the liver, spleen and kidneys

Whether the mere mechanical effect of the liquid in the pericardial sac contributed to the small flow in Albert H at the second examination cannot of course be determined, although it is likely enough that this was a factor in addition to the weakening of the myocardium and the insufficiency of the valves However, if the clinical diagnosis in the next case (Roman B) be accepted, liquid may be present in considerable amount in the pericardial sac without reducing the hand flow very markedly This patient was a man aged 22, in whom the diagnosis of mitral insufficiency and pericardial effusion was made Blood-flow measurements in the hands were obtained on three occasions, the first on May 29, 1912, when the heart dulness was much diminished and the

TABLE 17.—CALORIMETRIC MEASUREMENTS IN SECOND EXAMINATION OF ALBERT H

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
2 08	29 81	29 72		2 28	29 66	29 59	
2 10	29 74	29 67		2 29	29 675	29 61	
2 11	29 72	29 65	22 7	2 30*	29 685	29 62	
2 12	29 705	29 635		2 31	29 68	29 61	
2 13	29 695	29 625		2 32	29 69	29 625	
2 14	29 69	29 62		2 33	29 695	29 63	
2 15	29 68	29 61		2 34	29 70	29 63	
2 16	29 67	29 60	22 5	2 35	29 705	29 635	
2 17	29 665	29 595		2 36		29 63	
2 18	29 65	29 58		2 37		29 635	23 5
2 19	29 64	29 575		2 38		29 635	
2 20	29 635	29 565	22 6	2 39		29 645	
2 21	29 63	29 56		2 40		29 65	
2 22	29 625	29 555		2 41		29 655	
2 23	29 62	29 55		2 42		29 655	
2 24	29 62	29 55		2 43		29 66	
2 25	29 63	29 56		2 44		29 665	
2 26	29 64	29 57	22 7	2 45		29 67	
2 27	29 65	29 58		3 03½	29 41	29 49	

* Here the bringing in of the warm water attracted his attention In excitable patients this may cause a change in the flow

swelling of the legs present on admission had disappeared The flow came out 7 61 grams per 100 c c per minute in the right hand and 10 59 grams in the left hand, with room temperature 22 C He seemed fairly comfortable and had no dyspnea On May 31, when the weather was warmer, the room temperature being 26 3 C , the flow was 8 76 grams for the right hand and 11 05 grams for the left The average for the two hands is normal, or nearly so, and greater of course at the second examination, in accordance with the increased room temperature He left the hospital and after working in a foundry for two days, his legs became swollen and he had to return to the hospital on June 11 with the same

symptoms of broken compensation which he had at first, dyspnea, cough, greatly increased area of cardiac dulness and the edema already mentioned On June 13 his hand flow was again examined, and came out only 5 52 grams per 100 c c per minute for the right hand and 8 74 grams for the left, with room temperature 21 8 C , a considerable smaller flow than at the first examination, although the external temperature was practically the same The flow, then, concomitantly with the cardiac breakdown, has been decidedly diminished The marked inequality in the flow in the two hands persists throughout, although the relative difference is greater at the third than at the other two examinations No local difference in the hands or arms could be discovered to account for the difference in the flows It is possible that it is connected with intrathoracic changes not taken account of in the diagnosis The question of differences of flow in the two hands associated with unilateral changes in the lungs and pleurae will be taken up in a future paper

Roman B , a laborer, aged 22, was admitted to the City Hospital May 17, 1912 He had rheumatism for the first time three years before admission He has a typical cardiorenal facies He seems fairly comfortable, with no dyspnea The heart presents a pear-shaped area of dulness, left border in the mid-axillary line, right border one to three fingers' breadth to the right of the mid-sternal line, making an obtuse cardiohepatic angle The cardiac dulness is increased when he leans forward There is a loud systolic murmur at the apex The second sound in the pulmonic area is split The sounds are distant Pulse regular in rhythm, of fair volume, rather rapid rise, and well sustained Liver three fingers' breadth below costal margin, hard, sharp edge, not tender Edema of legs Urine negative

TABLE 18 —Calorimetric Measurements in Case of Roman B

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
3 21	31 03	31 05	21 4	3 32	31 52	31 68	
3 23	31 08	31 09		3 33	31 56	31 74	22 2
3 24	31 12	31 15	21 5	3 34	31 58	31 77	
3 25	31 19	31 23		3 35	31 60	31 825	22 6
3 26	31 23	31 315		3 36	31 64	31 89	
3 27	31 28	31 36		3 37	31 68	31 94	22 8
3 28	31 30	31 42		3 38	31 705	31 98	
3 29	31 36	31 47	21 0	3 39	31 735	32 025	22 5
3 30	31 42	31 56		3 52	31 555		
3 31	31 48	31 64	21 5	3 52½		31 83	

Cooling of calorimeters, R , 0 18 degree in thirteen minutes, L , 0 195 degree in thirteen one half minutes Volume of right hand 454 c c of left hand 439 c c He is right handed Water equivalent of calorimeters and contents, R , 3,458, L , 3,446 Pulse (sitting) 96 Rectal temperature 37 95 C

May 25, 1912, left cardiac border just outside the nipple line Right border one finger's breadth outside of sternal margin Cardio hepatic angle less obtuse Liver one finger's breadth below costal border Heart sounds louder and more distinct

May 29, 1912, the flow in his hands was measured Hands in bath at 3 12 p m , in calorimeters at 3 21¾, and out of calorimeters at 3 39 Mouth temperature 37 25 C

Second examination May 31, 1912 He says his right ankle pains him now It was painful three years ago when he had rheumatism for the first time Hands in bath at 1 51 p m, in calorimeters at 2 01½, and out of calorimeters at 2 20

The weather was considerably warmer than at the last examination

TABLE 19 —CALORIMETRIC MEASUREMENTS AT SECOND EXAMINATION OF ROMAN B

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
2 01	31 04	31 03	26 2	2 12	31 52	31 65	26 2
2 03	31 04	31 06		2 13	31 57	31 71	
2 04	31 08	31 125	26 3	2 14	31 61	31 77	26 3
2 05	31 125	31 17		2 15	31 67	31 85	
2 06	31 20	31 25	26 4	2 16	31 72	31 925	26 3
2 07	31 27	31 34		2 17	31 76	31 97	
2 08	31 32	31 42	26 4	2 18	31 795	32 025	26 2
2 09	31 38	31 48		2 19	31 83	32 08	
2 10	31 42	31 54	26 3	2 20	31 88	32 13	26 2
2 11	31 48	31 60		2 32	31 76	32 02	

Cooling of calorimeters in twelve minutes, R , 0 12 degree, L , 0 11 degree Volume of right hand 451 c c , of left hand 445 c c Water equivalent of calorimeters with contents, R , 3,456, L , 3,451 Pulse (sitting) 87 Rectal temperature, 37 65 degrees

June 12, 1912, he was readmitted to the hospital, complaining of precordial pain, dyspnea, and cough The right leg pains him more than before Lips cyanosed Finger tips bluish Edema in legs There is a diffuse wave during systole not prominent in any one region Limits of cardiac dulness were as follows

Interspace	Supine Posture Distance in Cm from Mid-sternal Line		Sitting Posture Distance in Cm from Mid sternal Line	
	Right Border	Left Border	Right Border	Left Border
6	9 5	16	12	17 5
5	8 0	15	9 5	15 5
4	6 5	10	6	11 0
3	4 0	7		

Auscultation, with moderate pressure of stethoscope, rough systolic murmur at apex Pulmonic second accentuated and split Aortic second, weak With pressure of stethoscope a soft blowing murmur is heard at apex during diastole The sounds are reduced in intensity Free fluid in abdomen

June 13, 1912, third examination of the hand flow Hands in bath at 1 58½ p m , in calorimeters at 2 09½, and out of calorimeters at 2 25

TABLE 20 — CALORIMETER MEASUREMFNTS IN THIRD EXAMINATION OF ROMAN B

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
2 09	31 145	31 06	21 4	2 20	31 345	31 47	22 0
2 11	31 13	31 07		2 21	31 37	31 51	
2 12	31 14	31 095		2 22	31 385	31 545	
2 13	31 165	31 13	21 8	2 23	31 395	31 59	22 1
2 14	31 18	31 17		2 24	31 405	31 625	
2 15	31 20	31 225	21 9	2 25	31 415	31 635	
2 16	31 22	31 26		2 34	31 28	31 51	
2 17	31 26	31 325	22 0				
2 18	31 29	31 375					
2 19	31 315	31 42					

Cooling of calorimeters in nine minutes, R, 0 135 degree, L, 0 125 degree Volume of right hand 451 c c, of left 443 c c Rectal temperature 37 3 C Water equivalent of calorimeters with contents, R, 3,456, L, 3,449

In a case (Robert T) diagnosed as pure aortic stenosis with perfect compensation in a man, aged 22 years, the average flow in the hands was at least equal to normal (10 45 grams per 100 c c per minute for the right hand and 13 97 grams for the left, with room temperature 20 3 C) He entered the hospital not for his heart condition, but for surgical treatment

Robert T, a railroad worker 22 years old, was admitted to the City Hospital Jan 23, 1913, for surgical tratment (crushed toes on left foot) He had typhoid fourteen years ago, also rheumatism in the neck for several weeks when a child Denies venereal diseases For four years after typhoid had shortness of breath and still gets short of breath if he has to hurry up, especially if he changes the

TABLE 21 —CALORIMETRIC MEASUREMENTS IN CASE OF ROBERT T

Time	Right	Left	Room Temp C	Time	Right	Left	Room Room C
2 52	31 22	31 13		3 00	31 38	31 42	20 3
2 53	31 19	31 10		3 01	31 42	31 48	
2 54	31 205	31 12	20 1	3 02	31 47	31 54	20 4
2 55	31 215	31 15	20 1	3 03	31 515	31 59	
2 56	31 23	31 18		3 04	31 56	31 65	
2 57	31 27	31 24	20 2	3 05	31 595	31 71	20 4
2 58	31 30	31 29	20 3	3 06	31 64	31 77	
2 59	31 35	31 36		3 27	31 28	31 41	

manner of his work, but not when he has become accustomed to a job and can work steadily without spurts He is well developed and well-nourished, no cyanosis Boundaries of cardiac dulness one and a half finger's breadth to left of nipple line, right sternal border, third interspace A blowing systolic murmur is heard over the entire area, but best in the aortic area where it is exceedingly

loud From time to time the murmur disappears for one beat, the apex beat becomes weak and the radial pulse vanishes, the murmur corresponding to the next beat being very loud, and the apex beat very strong The radial sphygmogram is the typical trace of aortic stenosis, with flat top and sloping up stroke The heart is slow The radial pulse shows fair volume, but to appreciate this the artery must be in a particular position as the tension is low and the arm fat The physical examination reveals nothing abnormal elsewhere The flow in the hands were measured Jan 31, 1913 Hands in bath at 2 41½ p m , in calorimeters at 2 52½, and out of calorimeters at 3 06 He sat during the whole examination with the injured left foot lying on a stool, the left leg horizontal and the right foot down (Table 21)

Cooling of calorimeters in twenty one minutes, 0 36 degree for R and L Volume of right hand 458 c c , of left 452 c c He is mostly right handed, he says Water equivalent of calorimeters with contents, R , 3,461, L , 3,456 Blood pressure, left arm, systolic 105 (palpation), 103 (stethoscope), 91 (sound sharply diminished), 87 (sound gone) Rectal temperature 36 6 C

The last cases to be described are two of mitral stenosis and insufficiency, one with good (Mary S) and the other with only slightly broken compensation (Frank W) at the time of the examination In the first case, a woman 26 years old, the flow works out at 15 74 grams per 100 c c per minute for the right hand and 14 26 grams for the left Even taking into account the relatively high room temperature (26 7 C) and the youth of the patient, these flows are, if anything, above the normal



Fig 3 —Tracings from Mary S

When the blood-flow in the hands is not below the normal we are usually justified in assuming that the output of the ventricles is not less than normal The large flow in this patient agrees well with the obviously exaggerated force of the ventricular stroke, and the absence of venous engorgement, of edema and of cyanosis It confirms the conclusion that the increased respiratory rate at the time of the examination is a nervous affair not depending on deficient exchange in the lungs Specimens of polygraph tracings are given in Figure 3 The *a* wave is prominent

Mrs Mary S , married woman, aged 26, a Hebrew, was admitted to the City Hospital May 29, 1913, complaining of pain in the chest, weakness and shortness of breath Seven months before admission she had an attack of acute articular rheumatism and during this attack she began to have pain in her breast Since then there have been several short periods during which she got short of breath There seems, however, to be a strong neurotic element in the case, and she does not look particularly ill nor does she show any marked dyspnea She is fat and well nourished No cyanosis, and little, if any, edema of the legs No venous engorgement, no pulsation in the neck The thorax is resonant throughout The breathing everywhere vesicular The apex beat is in the fifth interspace below the nipple, upper border of cardiac dulness in third interspace, right border, at right sternal margin, left border two finger's breadth out Auscultation showed the signs of mitral stenosis and insufficiency The

rather rapid breathing appears to depend largely on nervous excitement It is certainly not associated with any signs of deficient oxygen intake The lips are red and she is even more comfortable lying down with her head on a single pillow than sitting up

The blood-flow in the hands was estimated June 13, 1913 The day was rather warm (external temperature about 80 F) Hands in bath at 2 28 p m , in calorimeters at 2 40 and out of calorimeters at 2 52 Mouth temperature (two observations) 36 6 C Pulse (sitting) 84

TABLE 22.—CALORIMETRIC MEASUREMENTS IN CASE OF MARY S

Time	Right	Left	Room Temp C	Time	Right	Left	Temp Room C.
2 39	31 51	31 52		2 47	31 86	31 81	26 7
2 41	31 55	31 57	26 4	2 48	31 90	31 84	
2 42	31 60	31 60		2 49	31 93	31 87	26 8
2 43	31 65	31 64		2 50	31 97	31 90	
2 44	31 71	31 68	26 5	2 51	32 01	31 94	
2 45	31 76	31 73		2 52	32 06	31 99	26 8
2 46	31 80	31 76		3 07	31 89	31 83	

Cooling of calorimeters in fifteen minutes, R , 0 17 degree, L , 0 16 degree Volume of right hand 280 c c , of left 259 c c She is right-handed Blood-pressure, left arm, systolic 114, 95 (second phase), 62 (fourth phase), which was with practically no further fall in the pressure succeeded by the fifth phase (cessation of sound) Water equivalent of calorimeters with contents, R , 3,319, L , 3,302

The last case is that of Frank W , a man 34 years old, with mitral stenosis and insufficiency with somewhat broken compensation, which was rapidly restored Three examinations of the hand flow were made The first on the day of admission, before any treatment had been given, showed a flow of 15 20 grams per 100 c c per minute for the right hand and 15 29 grams for the left, with the unusually high room temperature of 30 9 C At the second examination, three days later, when the patient's clinical condition had improved, the flows were 11 95 grams for the right and 11 55 grams for the left hand, with the lower but still high room temperature of 28 5 C The third examination, made six days after the second, with a room temperature (30 8 C) the same as that during the first examination, showed a flow in the right hand of 11 44 grams per 100 c c per minute, and a flow in the left of 11 20 grams The patient was feeling well The re-establishment of compensation in this case was associated, then, with a diminution in the hand flow of 25 per cent This diminution in the hand flow coincided with a reduction in the pulse-rate, quieter action of the heart and an improvement of the patient's clinical condition It must be noted that the loss of compensation when the patient was admitted was not great, that his heart is permanently large and that there are no signs that the myocardium is, as yet, greatly

impaired The irregularity of the heart noted in the protocol is associated with premature auricular beats (Fig 4) This condition is not included among the graver cardiac irregularities by those who have specially studied the clinical disorders of the mechanism of the heart beat Loss of cardiac, or to speak more accurately, of cardiovascular compensation as expressed in an abnormal distribution of the blood, need not be always associated with a diminished flow through the periphery Doubtless the abnormal distribution of the blood, resulting in engorgement of the venous side of the vascular system and a rise of venous pressure, is harmful, and is going eventually to cripple the machine Yet it is not difficult to see that for a while the rate of the blood-flow through a part like a hand, far from being diminished, may be increased provided the driving power of the left ventricle, the aortic valves being intact, does not fail For the rise of venous and therefore of capillary pressure may, up to a certain point, by increasing the caliber of the small vessels, favor the flow of blood In order that this may result in increased flow through the part it is obvious that the arterial pressure must also be increased so that

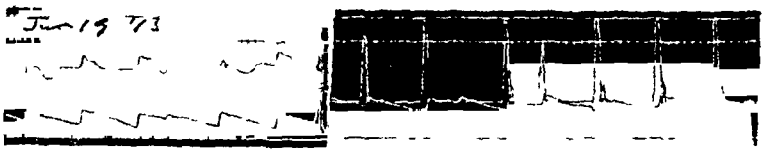


Fig 4.—Tracings from Frank W The left hand portion of the figure shows simultaneous tracings from the jugular bulb (upper trace) and the radial artery (lower trace) In the right hand portion of the figure simultaneous tracings from the apex (the large elevations) and the radial are given

a sufficient slope may be maintained And as a matter of fact, in Frank W, both the systolic and diastolic pressures in the brachial were materially higher at the first than at the second and third examinations Corroborative evidence for the conclusion that some loss of compensation when the myocardium is not seriously affected is not incompatible for a time with a fully normal hand flow, is found in the observations on Mary S and P B, already referred to

Frank W, an electrician aged 34, was admitted Feb 9, 1912, to Lakeside Hospital He says he never had rheumatism His illness began six weeks before admission with pain all over, especially in the lumbar region and then in the epigastrium In about a week shortness of breath came on and palpitation of the heart and he became blue in the face A few days before admission edema was observed in the lower eyelids and the feet The physical examination showed distended jugular veins with a systolic pulse Heart Apex beat in anterior axillary line in fifth interspace Here there is a systolic impulse and thrill followed by a diastolic impact A systolic impulse over the conus arteriosus Left border of cardiac dulness is beyond the anterior axillary line, 18 cm from the mid sternal line, and the right border 9 cm to right of midsternal line The

auscultatory signs are those of mitral stenosis and insufficiency The pulse is irregular and arhythmic Systolic blood-pressure 145, diastolic 108 Systolic pulsation of the liver Liver dulness extends to umbilicus in right nipple line Some edema of lower part of the back Legs and feet edematous When dependent, hands and feet are cyanotic No clubbing of fingers After a few days' rest the most prominent murmur became the presystolic rumble, heard best at the extreme apex, and indistinct elsewhere The systolic sound was followed by a short blowing murmur, heard best to right of apex The size and shape of the heart did not change materially The edema rapidly disappeared On March 6 the patient walked out of the hospital feeling well and with good cardiac compensation The pulse rate which was 160 on admission gradually fell, and varied from 70 to 80 at the time of discharge The minimum was 58 He broke down again after a time and was admitted to the City Hospital with the same symptoms and physical signs and was discharged improved He was readmitted June 16, 1913 He worked for three days before he came in but was losing appetite and becoming short of breath Some edema of the feet and ankles, but not a great deal Slight cyanosis and dyspnea only on exertion The point of the nose is red The boundaries of the cardiac dulness are practically the same as those given in the physical examination at Lakeside Hospital Pulsation in the neck veins

The hand flow was measured June 16, 1913, a few hours after his admission and before he had had any drug treatment The day was very warm Pulse (sitting) 72, irregular Hands in bath at 3 11½ p m , in calorimeters at 3 22¼, and out of calorimeters at 3 33

TABLE 23.—CALORIMETRIC MEASUREMENTS IN CASE OF FRANK W

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
3 22	31 49	31 49		3 29	32 09	32 08	
3 23	31 56	31 55	30 7	3 30	32 18	32 17	
3 24	31 64	31 64		3 31	32 27	32 255	
3 25	31 72	31 72	30 8	3 32	32 355	32 34	31 0
3 26	31 82	31 82		3 33	32 44	32 43	
3 27	31 90	31 90	30 9	3 49	32 37	32 36	
3 28	31 995	31 99					

Cooling of calorimeters in sixteen minutes, 0 07 degrees

Volume of right hand in calorimeter 456 c c, of left hand 452 c c Water equivalent of calorimeter and contents, R, 3,460, L, 3,456 Blood-pressure in left brachial 133 (systolic), 103 (change in sound) Another observation 133 (systolic), 133 (change in sound) The pulse was slow and the sound diminished very rapidly to extinction after the change was heard Rectal temperature 37 55 C

Second examination June 19, 1913 He says he feels better He has been receiving digitalis There is no edema of the ankles, no dyspnea, no distention of the neck veins, no redness of the nose The day was hot, but not so hot as that of the first examination Hands in bath at 1 55 p m , in calorimeters at 2 10¼, and out of calorimeters at 2 24

Cooling of calorimeters in twenty-three minutes, R, 0 18 degree, L, 0 16 degree Radial pulse (sitting) 51 Volume of right hand in calorimeter 450 c c, of left 439 c c Water equivalent of calorimeters with contents, R, 3,455,

L, 3446 Rectal temperature 37 75 C Blood pressure left arm, systolic 104, 84 (change in sound), the sound disappeared very soon after this reading Another observation, systolic 108, 88 (change in sound followed almost at once by its complete disappearance) Pulse (sitting) 60, counted with stethoscope over apex beat There is still arhythmia On holding his breath the heart rate suddenly increased considerably

TABLE 23.—CALORIMETRIC MEASUREMENTS ON SECOND EXAMINATION OF FRANK W

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
2 10	31 56	31 47		2 18	32 09	32 02	28 3
2 11	31 61	31 53		2 19	32 165	32 08	
2 12	31 685	31 60	28 7	2 20	32 215	32 135	28 4
2 13	31 76	31 68		2 21	32 295	32 20	
2 14	31 83	31 745	28 6	2 22	32 33	32 255	28 4
2 15	31 895	31 82		2 23	32 39	32 305	
2 16	31 97	31 89	28 5	2 24	32 465	32 36	
2 17	32 02	31 955		2 47	32 285	32 20	

Third examination, June 25, 1913 He feels well Appetite fair, but does not sleep well Since last examination the pulse rate has varied between 60 and 72 The respiratory rate has rarely exceeded 20 The temperature has not exceeded 98 4 F The day was very hot, about the same as the day of the first examination He says his heart thumps much less than when he first came in although it gives an occasional thump Hands in bath at 1 53 p m, in calorimeters at 2 04¼, and out of calorimeters at 2 19

TABLE 24.—CALORIMETRIC MEASUREMENTS ON THIRD EXAMINATION OF FRANK W

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
2 04	31 77	31 73		2 13	32 32	32 26	30 85
2 05	31 78	31 74		2 14	32 39	32 33	
2 06	31 83	31 80	30 6	2 15	32 44	32 38	
2 07	31 89	31 85		2 16	32 50	32 44	30 85
2 08	31 975	31 935	30 8	2 17	32 565	32 49	
2 09	32 04	31 995		2 18	32 62	32 555	30 85
2 10	32 12	32 06	30 85	2 19	32 70	32 63	
2 11	32 19	32 14		2 35	32 62	32 555	
2 12	32 26	32 20					

Cooling of calorimeters in sixteen minutes, R, 0 08 degree, L, 0 075 degree Volume of right hand in calorimeter 443 c c, of left 432 c c Water equivalent of calorimeters with contents, R 3,449, L, 3,440 Blood-pressure, left arm, systolic 104, 82 (change of sound followed very soon by its disappearance) Another observation Systolic 109, 86 (change of sound) Pulse (sitting) 60, counted with stethoscope over apex Respiration (sitting) 18 Rectal temperature 38 0 C

SUMMARY

1 The smallest flows observed in the series of cases dealt with in this paper have been associated with marked irregularity of the heart, indicating involvement of the mechanism of the heart beat In some of these cases the cardiac irregularity was associated with valvular lesions, but in those with the very poorest hand flows there was no evidence that the valves were involved

2 Three cases were diagnosed as auricular fibrillation, all associated with mitral stenosis (and insufficiency) While the flow in all was below the normal it was decidedly greater in one of the patients than in the other two, although he was much the oldest of the three This corresponded well with his clinical condition, which was good at the time of the examination, better than that of the other two patients The ventricle in spite of its irregular action was on the whole beating fairly strongly, and was obviously delivering on the average of its beats, a fair amount of blood to the periphery

3 In a case of mitral stenosis and insufficiency and tricuspid insufficiency, in which the heart showed marked arhythmia in part associated with the respiration (sinus arhythmia) the hand flow was also much below the normal

4 Abnormally small flows were seen in a number of cases diagnosed as myocarditis with regular action of the heart, but feeble heart sounds not accompanied by murmurs

5 In some cases diagnosed as myocarditis without valvular lesion, in which a serious clinical condition had previously existed, very fair hand flows, not much inferior to the normal, were found when the clinical condition had markedly improved

6 In several cases of mitral insufficiency with good compensation and no signs of impairment of the myocardium, the hand flow was found to be quite up to the normal amount In one case of mitral stenosis and insufficiency the flow appeared to be even somewhat greater than normal as if the big, well-compensated heart were discharging more than the usual quantity of blood

7 In a case of mitral insufficiency with broken compensation, venous engorgement and edema, but no dyspnea or cyanosis, the hand flow was quite up to normal In another case with the same lesions where there was little edema, no shortness of breath except on exertion, and none at the time of examination, the flow was also little if at all inferior to the normal flow, and the re-establishment of compensation was associated with an actual moderate diminution in the hand flow

8 In a case of mitral insufficiency with physical signs of pericardial effusion a flow not much inferior to the normal was found after the patient had been some time in the hospital and had regained compensation, and when the heart dulness had decidedly diminished When the patient, two days after leaving the hospital, broke down again and

TABLE 25.—DATA CONCERNING

Case	Age	Date	Pulse-Rate	Blood-Pressure	Temperature (C) of				Volume of Hands in c c	
					Room	Art. Blood	Calorimeters		Right	Left
							Right	Left		
Jacob W	59	5/ 1/11	100	190, (110)	25 0	37 1	29 92	29 93	508	486
					25 1		30 16			
					25 1		30 38			
James M	60	6/27/12	87	152, 72	23 0	37 15	30 93	30 93	368	356
N P	27	5/12/13	70	95	23 8	36 3	31 44	31 33	298	276
Jacob S	44	4/25/11	104		22 2	36 25	29 69	29 62	390	378
					22 6			29 76		
					22 7			29 78		
					22 7			29 83		
T F K	57	5/19/13	120	112, 58	24 0	36 85	31 76	31 75	444	458
Peter B	59	4/21/11	80		24 5	36 45	30 17	30 14	360	320
					24 3		30 50			
					24 3		30 79			
Peter B		5/17/11	84		26 6	36 5	30 63	30 43	410†	394†
					26 8		30 80			
					26 8		31 12			
					26 7		31 46			
John T	28	4/ 9/12	100	122	24 4	37 45	31 36	31 44	450	438
					24 3		31 39			
James H	63	5/29/13	70	134, 68	23 4	36 1	31 00	30 92	418	396
		6/16/13	57	110, (80)	30 9	37 1	31 69	31 74	408	392
Joseph Z	16	7/14/11	140		28 1	38 3	30 10	30 10	406	412
					28 2		30 51			
					28 3		30 85			
Mat K	42	1/24/11	80	170	22 8	36 6	29 72	29 40	440	435
P B	31	5/12/11	100		25 5	36 9	30 76	30 68	510	480
					25 8		31 55			
					25 5		32 13			
E M	14	1/26/11	116		23 4	38 0	30 14	30 07	255	240
Frank C	17	12/19/10	116		22 4	37 3	30 19	30 10	275	250
Albert H	31	4/ 9/12	120	160	24 7	38 6	30 95	30 92	447	450
Albert H		4/19/12	102	130	22 6	37 2	29 65	29 58	475	505
Albert H					23 8			29 65		
Roman B	22	5/29/12	96		22 0	37 45	31 41	31 52	454	439
Roman B		5/31/12	87		26 3	37 15	31 46	31 60	451	445
Roman B		6/13/12			21 8	36 8	31 28	31 36	451	443
Robert T	22	1/31/13	70	103, (91), 87	20 3	36 1	31 44	31 48	458	452
Mary S	26	6/13/13	84	114	26 7	36 6	31 81	31 78	280	259
Frank W	34	6/16/13	72	133, (108)	30 9	37 05	32 00	31 99	456	452
Frank W		6/19/13	60	106, (86)	28 5	37 25	32 04	31 95	450	439
Frank W		6/25/13	60	106, (84)	30 85	37 5	32 24	32 19	443	432

* Good compensation

† As his hands were short the marks were put higher up on the wrists than at the previous

‡ Badly broken compensation. The results on Stephen F., Mrs. A. and J. S. W. are sum

Heat Given Off in Gram-Calories			Blood-Flow in Gm Per Min		Flow Per 100 c c of Hand Per Min		Notes
ght	Left	In Mins	Right	Left	Right	Left	
050	801	13	12 50	9 55	2 46	1 96	Myocarditis
225		10	19 62		3 86		Left hand in water at 42 9 C
805		7	19 03		3 75		Left hand in water at 11 5 C
366	270	10	7 32	4 82	1 99	1 35	Senile heart Arhythmia
283	216	11	5 88	4 39	1 97	1 59	Auricular fibrillation
545	238	11	7 69	3 63	1 97	0 96	Auricular fibrillation
	170	5		5 82		1 54	Right hand in water at 43 3 C
	340	4		14 59		3 86	Right hand in water at 43 3 C
	340	5		11 76		3 11	Right hand dried and wrapped
,138	1,336	9	27 60	32 34	6 21	7 06	Auricular fibrillation *
,180	980	9	23 19	19 17	6 44	5 99	Myocarditis
,636		10	30 51		8 47		Left hand in water at 42 9 C
913		7	25 62		7 12		Left hand in water at 8 C
2,156	1,605	10	40 81	29 38	9 95	7 46	
924		5	36 03		8 79		Five min just preceding vasomotor test
1,643		10	33 93		8 28		Left hand in water at 8 C
1,130		9	27 69		6 75		Left hand in water at 43 C
516	396	10	9 41	7 32	2 09	1 67	Mitral insufficiency ‡
362		8	8 29		1 84		Left hand in water at 42 7
154	119	10	3 35	2 55	0 80	0 64	Mitral stenosis, insufficiency, tricusp insufficiency, arhythmia.
1,454	1,214	10	29 86	25 16	7 31	6 41	
1,505	1,165	8	25 50	19 75	6 28	4 79	Aortic insufficiency and stenosis ‡
1,402		9	22 22		5 47		Left hand in water at 8 C
1,197			22 31		5 49		Left hand in water at 43 C
2,159	1,704	11	31 70	23 90	7 20	5 49	Aortic insufficiency and stenosis
3,608	3,618	10	65 29	64 63	12 80	13 46	Mitral and tricusp insufficiency
2,627		11	49 60		9 72		Left hand in water at 8 C
2,102		9	54 40		10 67		Left hand in water at 43 1 C
2,988	2,772	13	32 49	29 88	12 74	12 45	Mitral insufficiency
2,378	2,331	12	30 96	29 97	11 25	11 58	Mitral stenosis and insufficiency
1,380	1,555	11	18 22	20 45	4 08	4 54	Mitral and tricusp insufficiency ‡
452	455	7	9 50	9 48	2 00	1 88	
	472	10		6 94		1 37	Right hand in water at 42 7 C
3,008	3,970	16	34 58	46 49	7 61	10 59	Mitral insuff with pericardial effusion
3,440	4,176	17	39 5	49 18	8 76	11 05	
1,610	2,466	13	24 92	38 74	5 52	8 74	
2 007	2,626	10	47 85	63 15	10 45	13 97	Aortic stenosis
2,091	1,763	11	44 09	36 94	15 74	14 26	Mitral stenosis and insufficiency
3 152	3,148	10	69 35	69 11	15 20	15 29	Mitral stenosis and insufficiency
3,280	3,146	13	53 80	50 73	11 95	11 55	
3 359	3,237	14	50 68	48 38	11 44	11 20	

examination

marized in a previous paper (Heart, 1911, iii, 84)

returned with some dyspnea, cough and swelling of the legs, the hand flow was found distinctly below its previous value

9 In two cases of mitral insufficiency with badly broken compensation, with venous engorgement, edema, cyanosis and dyspnea, the hand flow was much below the normal In one of these cases a striking diminution occurred a few days before death

10 In a case of apparently pure aortic stenosis with perfect compensation, the hand flow was normal in amount

11 In a case of aortic stenosis and insufficiency, with mitral insufficiency, while there were no signs of broken compensation, the hand flow was somewhat below the normal

12 In a case of aortic insufficiency and stenosis, with badly broken compensation (great dyspnea, pulmonary edema), the hand flow was greatly diminished

While it is obvious that some of the groups of cases are much better represented in the observations than others, as is easily understood, since they had to be collected as chance offered, certain general conclusions which, although of course at present only provisional, are yet entitled to some weight, seem to emerge with sufficient clearness It is scarcely necessary to say that in some points these conclusions simply confirm what is found by palpation of the radial pulse But it is by no means true that even the qualitative result of the blood-flow measurement can always be predicted from feeling the pulse

CONCLUSIONS

1 The hand flow is more apt to be markedly deficient in cases in which there is evidence of serious impairment of the myocardium, or more comprehensively and probably more accurately, of the mechanisms on which the force and rhythm of the heart's action depend, even when valvular lesions are absent, than in cases in which gross valvular lesions exist while the heart's action is strong and orderly This holds even when in valvular diseases clinical signs of loss of compensation are present In aortic insufficiency the hand flow appears to be less easily maintained at the normal level and more readily to fall below it when compensation is lost than in mitral insufficiency

2 Even where there is considerable venous engorgement in cardiac cases the flow in the hands may be little if at all diminished, provided that the myocardium is not impaired When in a case of broken compensation the flow in the hand remains good the reestablishment of compensation may be associated with a moderate decline

3 When in a cardiac case with broken compensation the hand flow is normal or not much diminished, the indication is that the myocardium has not as yet suffered serious impairment A decided diminution in the course of the case may be significant of impending failure of the myocardium

A STUDY OF THE BLOOD-PRESSURE IN CHILDREN SHOWING ORTHOSTATIC ALBUMINURIA *

MURRAY H BASS, M D, AND H WESSLER, M D
NEW YORK

In a previous communication[1] we have reported our findings in an examination of the size, shape and rate of the heart in a series of children showing orthostatic albuminuria The results of this examination revealed certain abnormalities in cardiac function and in the configuration of the heart, as seen with the fluoroscope As has been previously pointed out, many children who are the subjects of orthostatic albuminuria reveal clinically signs and symptoms of vasomotor instability which naturally lead one to inquire into the condition of the blood-pressure We have, therefore, made blood-pressure determinations on a number of these children previously examined in regard to their hearts, and here wish briefly to report our findings

Systolic and diastolic pressures were determined by the auscultatory method introduced by Korotkow, the stethoscope being held over the brachial artery at the elbow The instrument used was the one devised by Bendick,[2] the cuff, the same as the one used with the Janeway manometer, being 12 c c in width For the determination of diastolic pressure, that point was taken at which the sound heard over the artery suddenly becomes fainter This so-called fourth phase has been shown to correspond more accurately to the true diastolic pressure than the point at which all sound disappears[3] That the readings by the auscultatory method correspond well with those taken by older but more complicated methods has been proved,[4] but owing to its comparatively recent introduction, it has been but little used in determinations on children A short series of blood-pressure readings taken by the auscultatory method has been quite recently published by E Mueller,[5] but we have used as controls figures which we ourselves obtained in readings taken on a series of normal healthy boys ranging in ages from 10 to 15 years, whom we were enabled to examine through the kindness of Dr L B Rosenthal, to whom we here wish to express our indebtedness

* From the Children's Department of Mount Sinai Hospital Dispensary
* Submitted for publication August 2, 1913

1 Bass and Wessler Heart Size and Heart Function in Children Showing Orthostatic Albuminuria, THE ARCHIVES INT MED, 1913, xi, 403

2 Bendick A J Jour Am Med Assn, 1911, lvi, 1873

3 Warfield, L W THE ARCHIVES INT MED, 1912, x, 258

4 Warfield, L W Interstate Med Jour, 1912, xix, 856, also Goodman, E H, and Howell A A Univ Penn Med Bull, 1910-11, xxiii, 469

5 Mueller E Ztschr f Kinderh 1913 vii, Nos 3-4

Owing to the dependence of the albuminuria on the posture of the individual, the blood-pressure readings were taken both in the recumbent and in the upright positions, and as a further test of vasomotor competency readings were taken after a short period of exercise

The points to be considered then are the following

1 Do the blood-pressures (systolic, diastolic and pulse-pressures) of children showing orthostatic albuminuria differ in any way from those of normal children?

2 What is the effect of change of posture, recumbent or upright, on systolic, diastolic and pulse-pressure and how do these changes compare with those occurring in the normal child?

3 What is the effect of a short period of exercise (stair-climbing) on the blood-pressures?

4 Is it possible to correlate in any way the findings in the shape, size or rate of the heart before and after exercise with the blood-pressure reactions?

BLOOD-PRESSURE IN THE UPRIGHT POSTURE

Before proceeding to a consideration of our results, it may be well to review briefly the main findings of others in the examination of blood-pressures in normal children In general, it may be said that both systolic and diastolic pressure increase gradually according to the age of the child The height and weight of the individual, however, are factors of importance, for in two children of the same age the taller or heavier one will usually be found to have the higher pressure (Wolfensohn-Kriss[6]) Pressure also appears to be affected by the general hygienic surroundings, for Eminet[7] found it higher in children living under good conditions than in those dwelling in poor surroundings Sex does not appear to influence the pressure in children All authors are agreed that children show considerable variations in pressure, the same child giving different readings on different days though apparently under identical conditions Salle[8] found as great a variation as 18 mm of mercury, but others (Oppenheimer and Bauchwitz[9]) assert that 10 mm should be considered the normal limit of variation, while Mueller found a daily variation of only 2 to 5 mm Salle further points out that high readings frequently result from nervousness on the part of the children during examination

In comparing the results obtained by us in children showing orthostatic albuminuria with those obtained in normal children, we have considered it best to use as controls children of corresponding heights rather

6 Wolfensohn Kriss, P Arch f Kinderh, 1910, lii, p 332
7 Eminet P P Arch f Kinderh, 1907, xlvi, 328
8 Salle, V Jahrb f Kinderh, 1911, lxxiii, 273
9 Oppenheimer and Bauchwitz Arch f Kinderh, 1905 xlii, 415

than those of corresponding ages In Table 1 are tabulated the systolic diastolic and pulse-pressures of twenty-six children showing orthostatic albuminuria, and in parallel columns the figures for normal children of the same height The figures for the controls of this series were obtained from the literature and were recorded by means of the palpatory method This accounts for the fact that the figures for the diastolic pressures in the albuminurics seem abnormally low As a result of this the pulse-

TABLE 1—BLOOD-PRESSURES IN CASES OF ORTHOSTATIC ALBUMINURIA WITH NORMAL CONTROLS FOR THE CORRESPONDING HEIGHTHS

Orthostatic Albuminurics Blood-Pressure					Normal Controls* Blood-Pressure		
Case No	Height cm	Systolic	Diastolic	Pulse-Pressure	Systolic	Diastolic	Pulse-Pressure
1	115	125	65	60	80 95	70- 90	10 25
2	132	103	85	18	85-115	80-105	5-35
3	121	110	85	25	80-110	75-100	5 35
4	117	80	60	20	80- 95	70- 90	10 25
5	121	112	80	32	80-110	75-100	5-35
6	115	105	60	45	80- 95	70- 90	10 25
7	112	98	55	43	80- 95	70- 90	10 25
8	132	112	65	47	85-115	80-105	5-35
9	126	105	65	40	80 110	75-100	5-35
10	126	92	75	17	80-110	75 100	5-35
11	129	85	70	15	80-110	75-100	5 35
12	131	110	65	45	85-115	80-105	5 35
13	142	103	70	33	80 125	75-115	5-50
14	130	95	70	25	85-115	80-105	5-35
15	130	130	75	55	85-115	80 105	5-35
16	133	120	75	45	85-115	80 105	5 35
17	135	95	70	25	85-115	80-105	5 35
18	131	105	90	15	85-115	80-105	5 35
19	137	110	65	45	85-115	80-105	5 35
20	128	117	87	30	80 110	75-100	5 35
21	157	110	85	35	85-120	85-110	5 35
22	155	107	75	32	85 120	85-110	5 35
23	136	90	75	15	85-115	80-105	5-35
24	140	110	60	50	80-125	75 115	5-50
25	151	120	90	30	85 120	85-110	5-35
26	145	130	95	35	80 125	75-115	5 50

* These figures are from the literature and are obtained by the oscillatory and palpatory methods

pressures of these children are considerably greater than those of the controls That this is merely the result of a different method of obtaining the readings will be apparent when Tables 2 and 3 are considered, where the pressures in both the albuminurics and control children were taken by the same (auscultatory) method

The systolic pressures in eighteen of the twenty-six albuminurics fall within the variations found in health Eight of the cases, however, show

an increased systolic pressure, varying from 2 to 30 mm of mercury above the upper limit of the normal figures A careful reexamination of these eight children, however, failed to show that they differed in any way from the remainder, either in respect to subjective symptoms or in the configuration and size of their hearts As we pointed out in the previous paper before referred to, we are fairly sure that we have ruled out any cases of nephritis, and therefore do not believe that the increased systolic pressure is due to such disease

Pulse-pressure varies all the way from 15 to 60, the average being 33 A comparison between pulse-pressure and pulse-rate did not show any evidently constant relationship

We may, therefore, say that our results of pressure readings taken in the upright posture approximate closely to the figures for normal children We may also add that though the appearance of some of the children — their loss of vasomotor tone, as evidenced by cold, clammy skin, cyanotic hands and their generally visceroptotic habitus — would lead one to expect a subnormal blood-pressure, in not a single instance did we find this to be the case

The few figures for similar examinations to be found in the literature correspond with the results above stated Matthes,[10] who examined twenty-one boys 14 to 17 years of age, all of whom showed "albuminuria of puberty," found normal systolic pressures Salle,[8] in a very large number of blood-pressure determinations on various types of children, states that the few who were subject to orthostatic albuminuria did not differ in any way from the healthy ones Finally, Fischl[11] is also inclined to deny any great variation from the normal in the blood-pressures of children showing orthostatic albuminuria, though as he suggests, his readings being a little below the normal, this may be looked on as an evidence of vasomotor instability

BLOOD-PRESSURE ON CHANGE OF POSITION

In drawing conclusions from the behavior of the blood-pressure consequent on change of position from the recumbent to the upright or vice versa, it should be remembered that different results are obtained when the readings are made immediately after such change, than when some time has been permitted to elapse after the change of position With this in mind, our readings were not made until an interval of five minutes had elapsed to allow the circulation to adjust itself to the altered static conditions

In Table 2 are recorded the blood-pressures in both the upright and the recumbent positions A comparison of the figures shows that the

10 Matthes, M Deutsch Arch f klin Med, 1904 05, lxxxii, 605
11 Fischl, R Arch f Kinderh, 1909, lii, 261

following changes have occurred On standing up the systolic pressure fell in fifteen cases (average 12 5 mm), rose in six cases (average 4 mm), remained unchanged in five The diastolic pressure under the same conditions fell in eleven cases (average 7 mm), rose in ten cases (average 8 5 mm), remained unchanged in five cases The pulse-pressure fell in twenty-two cases (average 9 mm), rose in two cases (average 9 mm), and remained unchanged in two cases

ABLE 2—Blood-Pressures in Orthostatic Albuminurics in the Recumbent and Upright Positions

	Systolic			Diastolic			Pulse-Pressure		
Case No	Lying Down	Standing	Difference	Lying Down	Standing	Difference	Lying Down	Standing	Difference
1	140	125	—15	75	65	—10	65	60	— 5
2	103	103	0	85	85	0	18	18	0
3	105	110	+ 5	75	85	+10	30	25	— 5
4	90	80	—10	65	60	— 5	25	20	— 5
5	110	112	+ 2	85	80	— 5	25	32	+ 7
6	120	105	—15	70	60	—10	50	45	— 5
7	92	98	+ 6	60	55	— 5	32	43	+11
8	115	112	— 3	65	65	0	55	47	— 8
9	103	105	+ 2	60	65	+ 5	43	40	— 3
10	98	92	— 6	70	75	+ 5	28	17	—11
11	98	85	—13	70	70	0	28	15	—13
12	110	110	0	65	65	0	45	45	0
13	98	103	+ 8	58	70	+12	40	33	— 7
14	115	95	—20	85	70	—15	30	25	— 5
15	140	130	—10	75	75	0	65	55	—10
16	120	120	0	80	75	— 5	40	45	+ 5
17	110	95	—15	75	70	— 5	35	25	—10
18	115	105	—10	85	90	+ 5	30	15	—15
19	105	110	+ 5	55	65	+10	50	45	— 5
20	140	117	—23	100	87	—13	40	30	—10
21	130	110	—20	70	85	+15	60	25	—35
22	123	107	—13	80	75	— 5	43	32	—11
23	100	90	—10	77	75	— 2	23	15	— 8
24	112	110	— 2	55	60	+ 5	57	50	— 7
25	120	120	0	82	90	+ 8	38	30	— 8
26	130	130	0	85	95	+10	45	35	—10

It will be seen that in our cases of orthostatic albuminuria there is no constant change in systolic or diastolic pressures on change of position The pulse-pressure on the other hand with few exceptions was smaller in the upright position

For a critical consideration of these results it will be necessary to inform ourselves of the data in relation to this subject which are available in the literature Unfortunately, these disclose no unanimity of opinion, but are rather replete with contradictions Undoubtedly some of these discrepancies are dependent on the method of blood-pressure determina-

tion used by individual investigators This applies especially to diastolic pressures, which, when determined by the oscillatory method as has been heretofore the case, are subject to wide variation and make deductions based on them of doubtful value

Friedman[12] found that in the erect posture the systolic pressure falls A similar statement occurs in the reports of Shapiro[13] and Langowoy[14] With this, Hensen,[15] however, is not in accord, as he frequently failed to note such a change in pressure on standing Waldvogel[16] considers a drop in systolic pressure on rising as a normal reaction within certain quantitative limits When such a drop of pressure exceeds 10 mm of mercury it is considered by him pathologic Birtch and Inman[17] conclude that in healthy adults not in active training, the systolic pressure is slightly lower in the upright position, the diastolic pressure at the same time is slightly raised with the result that the pulse-pressure is smaller than in the recumbent position Dietlen[18] regards a slight systolic fall on rising as a normal change, a slight rise, however, not being necessarily pathologic In twenty-five normal persons he found that on change of posture in 22 per cent the systolic pressure was unchanged, in 22 per cent it was higher in the upright position, in 56 per cent it was lower in the upright position The diastolic pressure was usually increased, on an average 10 5 mm when the upright posture was assumed John,[19] in fifty-two cases, found the pulse-pressure on standing smaller in forty-nine and larger in three cases Quite recently Barach and Marks[20] have reported a series of cases in which pressure readings were taken on a large number of subjects both in the horizontal and in the upright positions, the experimenters attempting at the same time to eliminate the error caused by muscular exertion in changing from one posture to the other, by placing the subject on a movable platform capable of being tilted either vertically or horizontally From their findings these authors draw the following conclusions

When the element of muscular effort has been eliminated change of bodily posture from the erect to the horizontal will cause an increase in the maximum pressure, a decrease in the minimum pressure and an increase in the pulse pressure After five minutes in the horizontal posture when the subject is retilted to the erect posture the maximum pressure will diminish, the minimum pressure increase and the pulse pressure diminish It will be noted that in both instances the pulse pressure follows the same trend as the maximum pressure

12 Friedman Wien med Jahrb, 1882 (Quoted by Dietlen)
13 Shapiro Wratsch, 1881 (Quoted by Dietlen)
14 Langowoy Deutsch Arch f klin Med, 1900, lxviii, 268
15 Hensen Deutsch Arch f klin Med, 1900, lxvii, 436
16 Waldvogel Munchen med Wchnschr, 1908, No 32
17 Birtch and Inman Jour Am Med Assn, 1912, No 4, 265
18 Dietlen, H Deutsch Arch f klin Med, 1909, xcvii
19 John M Deutsch Arch f klin Med, 1908, xciii
20 Barach J H, and Marks, W L Arch Int Med, 1913, xi No 5, 485

Nearly all the subjects of this series respond in the same way The most notable exception was in the "poor muscular cases" These showed a tendency to reversal of the pressure curve From the erect to the horizontal caused in more than half the cases a decrease of the maximum pressure and an increase in the minimum pressure

We may, with certain restrictions, extract the following conclusions from these data

1 On change of posture, systolic pressure may rise or fall to a slight degree

2 In the upright posture diastolic pressure is slightly higher than in the recumbent

3 Pulse-pressure is smaller in the upright posture

Using the Korotkow method, we have taken pressure readings of twenty-two healthy boys between the ages of 10 and 14 years The results of this examination may be seen in Table 3 and may be summarized as follows

TABLE 3—INFLUENCE OF CHANGE OF POSTURE ON BLOOD-PRESSURES OF NORMAL CONTROL CHILDREN READINGS BY THE AUSCULTATORY METHOD

	Systolic			Diastolic			Pulse-Pressure		
Case No	Lying Down	Standing	Difference	Lying Down	Standing	Difference	Lying Down	Standing	Difference
1	117	120	+ 3	87	80	— 7	30	40	+10
2	105	102	— 3	77	78	+ 1	28	24	— 4
3	112	110	— 2	80	75	— 5	32	35	+ 3
4	100	112	+12	55	55	0	45	57	+12
5	138	140	+ 2	90	90	0	48	50	+ 2
6	135	130	— 5	80	70	—10	55	60	+ 5
7	123	123	0	73	73	0	50	50	0
8	130	120	—10	85	75	—10	45	45	0
9	165	175	+10	115	110	— 5	50	65	+15
10	140	130	—10	120	95	—25	20	35	+15
11	110	112	+ 2	82	90	+ 8	28	22	— 6
12	120	130	+10	60	75	+15	60	55	— 5
13	140	125	—15	75	87	+12	65	38	—27
14	112	120	+ 8	75	77	+ 2	37	43	+ 6
15	115	125	+10	60	70	+10	55	55	0
16	140	135	— 5	50	62	+12	90	73	—17
17	145	145	0	60	75	+15	85	70	—15
18	125	117	— 8	82	90	+ 8	43	27	—16
19	142	145	+ 3	95	85	+10	47	60	+13
20	120	115	— 5	85	90	+ 5	35	25	—10
21	162	142	—20	100	100	0	62	42	—20
22	130	125	— 5	85	80	— 5	45	45	0

In the upright position Systolic pressure is lower in eleven cases (average 8 mm Hg), is higher in ten cases (average 6 5 mm Hg), is unchanged in two cases Diastolic pressure is lower in eight cases

(average 11 mm Hg), is higher in eleven cases (average 10 mm Hg), is unchanged in four cases Pulse-pressure is smaller in nine cases (average 14 mm Hg), is larger in eight cases (average 9 mm Hg), is unchanged in four case

From these figures it will be seen that in these normal children change of posture resulted in no constant change of the systolic diastolic or pulse-pressures Any of these factors may vary in either direction

If it is assumed for purposes of argument that in orthostatic albuminuria there is some abnormality in the circulation, an assumption that is freely made by most investigators of this condition, one is not unnaturally encouraged to seek in variations in blood-pressure, especially in some alteration in pulse-pressure, some evidence of such an abnormality Especial impetus was given to this phase of the subject by the work of Erlanger and Hooker [21] These authors showed in their case of orthostatic albuminuria that the excretion of albumin followed closely a diminution of pulse-pressure resulting from a change of position from the recumbent to the upright When by various mechanical means such a drop of pulse-pressure was prevented, excretion of albumin failed to take place This association of fall of pulse-pressure with excretion of albumin led us to hope that in our cases we might find uniformly a decrease of pulse-pressure on change of position from the recumbent to the upright, or at least some evidence of unusual response to postural conditions Our results as already given, however, do not show any such marked fall of pulse-pressure on change of position, although the figures are somewhat smaller than the controls

This subject acquires an added interest from its relation to enteroptosis A majority of our cases show definite stigmata of this condition, probably of congenital origin or disposition Its significance lies in the abnormality in circulation and blood-pressure, which is supposed to result from an atonic condition of the abdominal wall Birtch and Inman,[17] in cases of visceroptosis with relaxation of the abdominal wall, concluded that on rising, although the systolic pressure may behave in a normal manner the diastolic pressure either remains unchanged or drops They attribute this drop of diastolic pressure directly to a loss of support of the splanchnic circulation due to atonic abdominal musculature

Such a reaction we have failed to find in our cases Whatever the effects we may attribute to muscular atony in orthostatic albuminuria, it may be affirmed that a definite change of peripheral systolic, diastolic and pulse-pressure is not one of them The muscular atony, if it has any effect at all in these cases, operates otherwise than by the production of dynamic changes in the peripheral circulation

21 Erlanger and Hooker Johns Hopkins Hosp Rep, 1904

In concluding this subject we wish to refer to the statement of Elliott,[22] that a drop of systolic pressure in rising is pathognomonic of cases of orthostatic albuminuria It is scarcely necessary to say that such a drop of pressure is a normal phenomenon in many cases, and that on the other hand it does not occur with sufficient regularity to invest it with the dignity of an essential symptom

BLOOD-PRESSURE AFTER EXERCISE

In the search for tests of heart efficiency in both organic and functional circulatory disturbances, the response of the circulatory apparatus to bodily exertion has at various times been used as an index of its capabilities In orthostatic albuminurics, it is true, there is no question of organic heart disorder in the ordinary sense We have indeed shown in a previous article,[1] that in many cases the hearts are undersized, abnormal in shape and in some cases have noted what may be regarded as an abnormal response on the part of the heart to increased demands on its energy Such changes are, however, probably congenital or rather developmental in origin, and their causes lie deeper and are more involved than the ordinary causes of heart disease It cannot be doubted, however, that functionally the cardiovascular system in these cases is not so efficient as in normal children, in proof of which witness the variety of subjective symptoms of which they so frequently complain, the commonest of which are dyspnea and palpitation occurring even after the slightest bodily exertion

In attempting to gather some information from the literature on the relationship between blood-pressure and exercise, we are at once confronted with a variety of opinions and numerous contradictory statements Some as for example, Stursberg and Schmidt,[23] believe that an examination of the blood-pressure after exercise is of prognostic significance, for they say that an individual, who, after very slight bodily exercise, shows a very large rise in blood-pressure, will be more easily harmed by excessive exercise than one in whom the pressure does not rise so high This is especially true of those who show marked irritability of their pressure and their pulse-rates In the same way Kleissel,[24] from experiments on soldiers, concludes that neurotic individuals reveal an increase of blood-pressure and pulse-rates after exercise to a degree not shown by normal persons As opposed to these views are the conclusions of Fantus and Staehelin,[25] who state that in healthy adults the regulation of the blood-pressure is too complicated for us to draw any conclusions

22 Elliott, A R Interstate Med Jour , 1911, xviii, 721
23 Stursberg and Schmidt Munchen med Wchnschr , 1913, No 4 p 174
24 Kleissel, R Wien med Wchnschr , 1911, No 28, 1810
25 Fantus and Staehelin Ztschr f klin Med , 1910, lxx, 444

from the behavior of the maximum and minimum pressures after exercise Masing,[26] as the result of pressure readings on numerous subjects, decides that in general the increase in blood-pressure after work is proportional to the work done, when the work is repeated the increase is not so great The increase after muscular exercise is in proportion to the mental effort employed, and the latter varies with practice In young individuals pressure remains high and fairly constant during work, whereas in the aged it drops after having attained its maximum Finally, Lowsley,[27] after conducting a series of very interesting experiments on athletes, taking systolic and diastolic pressure readings immediately and at intervals after exertion, concludes that the pressure reacts differently depending on the grade of the exercise — whether it be moderate, rapid, vigorous, fatiguing or exhausting He believes that prolonged exercise which causes an increase in the pulse-rate also causes an increase in systolic and diastolic pressures The systolic shows the greatest rise, hence there is an increase in the pulse-pressure which may be interpreted to mean that the heart-beats are augmented as well as accelerated He emphasizes, as an important gauge of the circulation, the time taken for the pulse-rate and blood-pressure to return to normal after exercise It is also of interest to note in this connection that Lowsley found albuminuria in those cases which showed small pulse-pressures

Our method of studying the effect of exercise on the blood-pressure was a follows The pressures were at first determined in the upright position at rest The children were then made to run up and down three flights of stairs rapidly The blood-pressure was then immediately taken again, never longer than one-half minute after cessation of the exercise The need of this haste in making the second reading lies in the well-known observation that the pressure falls very rapidly within a few minutes after the exercise Readings made after such an interval give quite different results

Summarizing Table 4, in which we have recorded the results of our blood-pressure determinations in children showing albuminuria we find that the systolic pressure rose in eighteen cases, was unchanged in two and fell in one case The increase in systolic pressure was as high as 35 mm Hg The diastolic pressure fell in thirteen cases, was unchanged in one and rose in seven cases The greatest fall was 15 mm , the greatest rise was 15 mm The pulse-pressure rose in every case after exercise, the rise varying from 10 to 50 mm

For comparison, we append in Table 5 the blood-pressure of our normal control children taken before and after exercise When these and

26 Masing E Deutschr Arch f klin Med , 1902, lxxiv, 253
27 Lowsley O S Am Jour Physiol , 1910 11, xxvii, 446

TABLE 4.—Blood Pressures in Orthostatic Albuminurics Before and After Exercise

Case No	Systolic			Diastolic			Pulse-Pressure		
	Before Exercise	After Exercise	Difference	Before Exercise	After Exercise	Difference	Before Exercise	After Exercise	Difference
1	105	100	— 5	65	45	—20	40	55	+15
2	103	105	+ 2	70	60	—10	33	45	+12
3	92	115	+23	75	90	+15	17	35	+18
4	130	130	0	95	90	— 5	35	40	+ 5
5	110	145	+35	85	70	—15	25	75	+50
6	95	113	+18	70	63	— 7	25	50	+25
7	80	100	+20	60	75	+15	20	25	+ 5
8	112	125	+13	80	80	0	32	45	+13
9	105	122	+17	60	68	+ 8	45	54	+ 9
10	85	120	+35	70	85	+15	15	35	+20
11	130	155	+25	75	90	+15	55	65	+10
12	103	106	+ 3	85	65	—20	18	41	+23
13	90	90	0	75	70	— 5	15	20	+ 5
14	120	135	+15	75	70	— 5	45	65	+20
15	110	113	+ 3	85	82	— 3	25	31	+ 6
16	95	125	+30	70	75	+ 5	25	50	+25
17	110	120	+10	65	67	+ 2	45	53	+ 8
18	98	117	+19	55	50	— 5	43	67	+23
19	110	115	+ 5	65	60	— 5	45	55	+10
20	117	125	+ 8	87	85	— 2	30	40	+10
21	120	155	+35	90	85	— 5	30	70	+40

TABLE 5.—Blood Pressures in Normal Control Children Before and After Exercise

Case No	Systolic			Diastolic			Pulse Pressure		
	Before Exercise	After Exercise	Difference	Before Exercise	After Exercise	Difference	Before Exercise	After Exercise	Difference
1	120	130	+10	80	65	—15	40	65	+25
2	102	135	+33	78	70	— 8	24	65	+41
3	110	127	+17	75	65	—10	35	62	+27
4	112	135	+23	55	65	+10	57	70	+13
5	140	165	+25	90	65	—25	50	100	+50
6	130	160	+30	70	60	—10	60	100	+40
7	123	150	+27	73	55	—18	50	95	+45
8	120	155	+35	75	90	+15	45	65	+20
9	175	175	0	110	95	—15	65	75	+10
10	130	140	+10	95			35		
11	112	138	+26	90	70	—20	22	68	+46
12	130	150	+20	75	75	0	55	75	+20
13	125	180	+55	87	100	+13	38	80	+42
14	120	160	+40	77	65	—12	43	95	+52
15	125	180	+55	70	70	0	55	110	+55
16	135	195	+60	62	70	+ 8	73	125	+52
17	145	160	+15	75	50	—25	70	110	+40
18	117	160	+43	90	82	— 8	27	78	+51
19	145	180	+35	85	70	—15	60	110	+50
20	115	150	+35	90	100	+10	25	50	+25
21	142	185	+43	100	90	—10	42	95	+53
22	125	170	+45	80	90	+10	45	80	+35

the orthostatic albuminuria cases are compared, the following points may be noted Both groups react qualitatively in the same manner, the systolic pressures in the orthostatic cases, however, do not increase as much as do those of the normal cases The diastolic pressures correspond fairly well in the two series The pulse-pressure after exercise in the cases of orthostatic albuminuria are much smaller than in the controls (as 17 44) This diminution is attributable to the difference in systolic pressures

SUMMARY

To sum up the result of our investigation we may say that the blood-pressures of children suffering from orthostatic albuminuria differ but little from those of normal children In spite of the apparent vasomotor insufficiency which many of these children showed, the blood-pressure reactions, both in the upright and recumbent postures and also after exercise, revealed no characteristic anomaly Nor were we able in any way to correlate the blood-pressure findings with the findings in regard to heart shape and size or pulse-rate Children with orthostatic albuminuria who showed marked cardiovascular symptoms could not be differentiated by means of blood-pressure tests from the remainder of the group

These results are disappointing, for the numerous interesting hypotheses concerning the etiology of orthostatic albuminuria would lead one to suppose that the vasomotor system must be in some manner to blame for the condition, especially in view of the results obtained by Erlanger and Hooker The relationship of lordosis of the lumbar spine to orthostatic albuminuria has also been brought into connection with an abnormal cardiovascular system, since it has been suggested that only those children react to lordosis by an excretion of albumin in whom the vasomotor system is unable to prevent congestion of the kidney This hypothesis appears plausible, but it cannot be proved or disproved by an examination of the pressure in the peripheral vessels All that can be said at present is that the blood-pressure in the brachial artery reacts normally, though whether or not this would apply to the blood-pressure in the renal vessels of the same case, we are unable to state

41 West Eighty-Third Street—122 West One Hundred and Eighteenth Street

EXPERIMENTS TO DEVELOP A MORE WIDELY USEFUL METHOD OF BLOOD-TRANSFUSION*

HENRY S SATTERLEE, M D, AND RANSOM S HOOKER, M D
NEW YORK

In the present discussion a comprehensive review of all methods of transfusing human blood would lead us too far from our immediate subject It will therefore be expedient to dismiss briefly and with very general consideration those methods of transfusion which involve the direct anastomosis of blood-vessels and not to attempt to estimate their relative merits For a detailed discussion of the difficulties and limitations of this class of operation the reader is referred to the notable work of Crile published in 1909 Since the introduction of Crile's method, other ingenious devices have been contrived to facilitate the accomplishment of transfusion by an end-to-end junction of the blood-vessels, and there have been numerous accounts of the success of these operations Less notice, however, has been given of the failures which from time to time have occurred From a study of the literature, and from the personal communications of those who have had experience with the different ways of performing transfusion by anastomosis, we believe that it is fair to say of such methods that they ought not to be attempted by the general practitioner, that even in the most skilful hands, the presence of some slight adverse condition may seriously interfere with the chances of success, and that in all of these methods there is no satisfactory way of measuring the amount of blood which is actually transfused

Passing on to those methods of transfusion which depend on the agency of an intermediate receptacle, one finds, in looking over the literature, that such methods were employed many years ago with defibrinated blood, particularly as applied to battle-field surgery Esmarch, in 1877, in his work on the treatment of the wounded in war, gave a detailed description of the method advocated by him for use in the Prussian army He believed in using only hydrostatic pressure to carry the blood into the vein, and the illustration which he gives of his contrivance for this purpose bears a very striking resemblance to a modern salvarsan infusion apparatus Another device of this period was Collin's transfusion apparatus, which consisted of a large funnel and a cylindrical force-pump, communicating by a check-valve with a glass reservoir From this reservoir, which also served as an air-trap, the blood was led by a tube

* From the Physiological Laboratory of the College of Physicians and Surgeons, Columbia University
* Submitted for publication Aug 8, 1913

and cannula into the vessel of the recipient Hasse about the same time employed a screw-piston syringe, and Uterhart a syringe designed to trap air-bubbles Hurter, another contemporary surgeon, recommended a similar method for arterial transfusion It is mentioned by Esmarch that injection into an artery of the recipient obviates the danger of overdistending the right heart, and that where a vein is used, not more than 25 gm of blood a minute should ever be injected

With all these methods the blood was carefully defibrinated by whipping, and Esmarch recommends two defibrinations and two filtrations to insure against the possibility of injecting coagulum The question of asepsis was at that time, of course, considered only in the most general way

About ten years later Esmarch, influenced by the researches of Kohler on the properties of defibrinated blood, came to the conclusion that there is a dissolving action of the fibrin ferment in defibrinated blood on the red blood-cells, and also the possibility of causing coagulative changes in the circulating blood of the recipient, and that therefore "every form of transfusion of blood is to be rejected" A few years later Kohler's work was confirmed by Edelberg and much later by Schultz In 1900 Esmarch reiterated his condemnation of defibrinated blood and recommended intravenous saline infusion for every indication that formerly demanded transfusion Fuld, in 1903, and Morawitz in 1905, showed that the harmful substances in defibrinated blood was changed to an apparently harmless modification if filtration from the coagula was conducted without pressure, and if the blood was kept for a short time after defibrination before being injected into the circulation

Those who have more recently experimented with the use of homologous defibrinated blood for intravenous transfusion vary in their estimate of the value of the procedure as a therapeutic method Morawitz, in a later communication, says that there is great danger in infusing defibrinated blood into the circulation unless ample time has been allowed for the completion of all changes which may take place in the coagulative factors The experiments of Moldovan indicate that thirty-five minutes after defibrination is the time-limit of safety Although Curtis and David have given intravenous injections of freshly defibrinated blood in dogs in four experiments and found it to have been safely utilized by the recipient animal, these authors are not led to advocate the method for human transfusion Hurter asserts his belief, based on clinical results in thirty-five transfusions for pernicious anemia, that the use of fresh unaltered blood is much better than the use of defibrinated blood, especially in the aplastic type of this disease The investigations of Pike, Guthrie and Stewart on the resuscitation of the blood, indicate that the preservation of defibrinated blood for twenty-four hours impairs the

physiological functioning of the erythrocytes, and they express the belief that blood, when preserved outside the circulation, soon undergoes changes which affect its nutritive as well as its bactericidal properties The experiments of Genou in 1901 apparently indicated that plasma had little or no bacteriolytic action *in vitro* on a variety of organisms which were destroyed by serum This supported Metchnikoff's theory that complement is derived from the leukocytes and liberated by changes induced in them during the process of coagulation Addis, however, has since shown that Genou did not directly compare plasma with serum, but serum from cell-free plasma with serum from blood, and concludes from his experiments with "paraffin plasma" which he obtained by a very careful technic, that complement is not derived from injured leukocytes, that there is as much complement in plasma as in serum, that it is probably present in the circulating plasma of the blood and is not a product of coagulation

Stevens and Lee, in 1884, in the course of perfusion experiments with defibrinated blood on the frog and terrapin, demonstrated the presence of an active vasoconstricting substance in defibrinated blood which was apparently due to some product of the coagulative process The experiments of Brodie were, however, the first to indicate that there were distinctly different vasomotor agents in plasma and in serum, the main result of his investigations being the discovery of a vasodilator substance in blood-serum acting centrally on the vasomotor system through the pulmonary branches of the vagus, which this author determined to be a protein of the albumin class, coagulable by boiling and produced during the process of clotting by some action of the corpuscles Sollmann found that serum produces a direct vasodilator effect on the isolated kidney limited to the efferent vessels, and O'Connor determined that the vasoconstricting effect of serum was due, not to its epinephrin content, but to some substance liberated during coagulation O'Connor's observations were later confirmed by Falta and Fleming, and the existence of such a substance was inferred by Stewart and Harvey from their finding that the serum from a patient with Addison's disease, whose adrenal remains after death yielded no suprarenin, produced as great a constrictor effect on the vessels of an isolated rabbit kidney as did normal serum

Stewart and Harvey followed up this observation with a series of perfusion experiments showing the effects of human, dog, rabbit and sheep serum and plasma on the isolated renal vessels of the dog and rabbit, on the peripheral vessels of the rabbit and frog, and on the coronary arteries of the isolated rabbit heart, and came to the conclusion that there is (a) a vasodilator substance in plasma and in serum which is specific for the vessels of the kidney, acting directly on the muscle coats of the arteries, which is a protein of the albumin class and is precipitated

by boiling and by alcohol, (b) a vasoconstrictor substance in serum, liberated by the process of clotting of the blood, which acts on the renal vessels and also on the vessels of the limb which is not a protein, is soluble in alcohol and resists boiling

If these conclusions be accepted as the vasomotor effects of plasma or undefibrinated blood and of serum or defibrinated blood, it is evident that the action of the latter must be a complex resultant of opposed forces

That a deleterious effect is produced by the presence in blood intended for transfusion of substances having such a complicated action on the vasomotor system, would be difficult to prove in any given case But, generally speaking, it is desirable beyond peradventure that blood for transfusion should be without any alteration of normal physiological function, except where some special therapeutic effect is sought, as from specifically immunized blood Apart from the dangers of insufficient defibrination, the chances of bacterial contamination, and the other objections just mentioned, the use of defibrinated blood seems particularly inadequate from a physiological standpoint in those cases (purpura, hemophilia, etc) in which the potential clotting elements of the patient's blood are presumably lacking or at fault

The transfer of fresh blood from one human being to another through the agency of an intermediate receptacle seems to have been first practiced by von Ziemssen over twenty years ago The method described by this author required one operator and three assistants The arm of the donor was congested by a bandage and a prominent vein at the bend of the elbow was punctured without skin incision by means of a hollow cylindrical needle and the blood was withdrawn into a 25 c c piston syringe While this syringe was filling another needle was likewise introduced into the recipient's vein The ligature was then removed from the recipient's arm and the first syringe, as soon as filled, was quickly carried over and attached to the needle in the recipient's vein and the blood slowly injected Meanwhile a second syringe was attached to the donor's needle as soon as disconnected from the first syringe, and the same procedure repeated A third syringe followed in the same way, the first one being meanwhile carefully washed out by an assistant Ziemssen reported that the transfer of from 250 to 300 c c of blood in this manner took not longer than fifteen to twenty minutes In his first series of seven cases he stated that he observed chills and elevations of temperature in three cases and that in one of these instances he had trouble with a clot in the recipient s needle which had to be removed He, however, considered the method less liable to the risk of causing dangerous coagulative changes in the circulating blood than transfusion by end-to-end anastomosis or the use of defibrinated blood

A method was recommended by Moritz in 1911 which did not materially differ from Ziemssen's, except that the needles were fitted with stop-cocks and connected with the syringe by means of an intermediate tube of glass and rubber also provided with stop-cocks Immediately after each withdrawal or injection of blood, sterile normal salt solution was injected through the needles and the stop-cocks were closed Strips of adhesive plaster were used to minimize as far as possible the movement of the needles while within the veins It is mentioned by Moritz that the needles are of such fine caliber that no clots of any size could possibly be injected into the circulation Hurter recommends Moritz's method and claims to have had success with it although he refers to it as involving a delicate technic

More recently Lindeman has advocated a method which he has practiced successfully on children and adults in a considerable number of cases His method differs from that of Ziemssen and of Moritz in that he has devised a special set of invaginated cylindrical cannulas, and that a dozen or more syringes of 20 c c capacity are employed in rapid succession to convey the blood from one cannula to the other An improvement over the Ziemssen and Moritz technics is the avoidance of traumatizing the intima of the veins by the many abrading movements within the blood-vessel of a sharp-pointed instrument, incidental to the frequent connection and disconnection of the syringes This feature is minimized by Lindeman in that the sharp innermost cannula of his set is withdrawn from the vein as soon as it has done its work of penetration It should also be mentioned that all cannulas receive an internal coating of liquid paraffin before use

As no special measures to delay or prevent clotting of the blood while in the receptacle are employed in this class of operation, success and safety necessarily depend on celerity of action in conveying the blood from the vessel of the donor to that of the recipient Prompt and repeated washing out of the cannulas with normal salt solution is also imperative Each syringeful of blood involves one interruption of flow, two connections to blood-vessels and two disconnections, which subjects the punctured vein-wall to a considerable amount of manipulation during the course of the transfusion Where much blood is to be transfused by this method many syringes are required, and this makes the outlay for apparatus a matter for consideration in judging of the general usefulness of the procedure To keep a dozen or more syringes in good working order with strict asepsis, and to have the intelligent, prompt and coordinated action of at least three operators, renders this type of operation impossible for emergency use except in a hospital, and takes it out of the hands of the general practitioner We believe, however, that this method in its simplest application, as reported by Cooley and Vaughan, should prove

a valuable resource in emergencies, where only 10 to 20 c c of blood are required, and an ordinary syringe with needle is the most convenient instrument at hand

It is evident to anyone who has given it consideration that a large field of usefulness would be open to the operation of blood-transfusion if it could be performed safely, quickly and surely by anyone possessing ordinary surgical skill, and, if possible, without expert assistance

It occurred to one of us that such a practicable method might be evolved if a satisfactory apparatus could be devised to withdraw a considerable quantity of blood at one time from a vessel of the donor and inject it into a vessel of the recipient without risk of clotting, and more particularly from a study of the recent investigations of Howell, of Duke and of Whipple on the nature and significance of the factors concerned in blood-coagulation, it seemed that there were two alternative lines of experimentation which held out some promise of overcoming the danger of clotting in blood-transfusion These alternatives are

(*a*) The employment of an intermediate system, providing no point of contact with any moistenable surface, and at the same time minimizing as far as possible the exposure of broken tissue surface to the blood stream, (*b*) the employment of a sufficient amount of some physiologic agent (antithrombin), to restrain or offset the initiative factors of coagulation during the time of the conveyance of the blood through a foreign system, such as glass and metal

The investigation of the possibilities of the second of these alternatives is already under way and will form the subject of a later communication

It is, however, with the development of the first of these procedures that the present paper has to do, and we may say at once that this has resulted in a method of drawing blood from the donor's blood-vessel directly into a paraffin receptacle, carrying the blood to the recipient, and injecting it into the recipient's circulation in precisely the same way in which it was obtained from the donor Excepting the air-contact of its upper surface while in the receptacle, the blood does not touch anything but paraffin during its passage The paraffin receptacle consists of a thin film of hard paraffin, lining a firm supporting structure The latter comprises a tubulated glass cylinder, closed at the lower end by an asbestos stopper, through which passes a metal cannula The upper end of the cylinder passes directly into an aspirating tube containing an air-filter and terminating in a mouth-piece

In considering the work which has already been done with paraffin-coated apparatus, we have been especially impressed with the reports of Curtis and David, and of Risley and Irving From the findings of these investigators it seemed wise to plan some preliminary experiments to ascertain the essential requirements of a conveying system with a protective lining

The materials which appear to have served best as anticoagulative coating agents in former experimental work[1] are the paraffins, ordinary commercial petrolatum, the neutral saponifiable oils and stearic acid The latter seemed to possess no peculiar advantage and was rejected by us on theoretic grounds, because of the possible hemolytic effect[2] which is known to be a characteristic of some of the unsaturated fatty acids The trial mixtures were, therefore, confined to ingredients of the first three classes Olive oil and the expressed oil of almonds were chosen from the neutral saponifiable oils, and, when combined in proper proportions with paraffin and petrolatum, formed fairly satisfactory mixtures The vegetable oils were, however, finally abandoned on account of their tendency to favor the growth of mold

In judging the suitability of the paraffin mixtures, the physical qualities considered were

1 Macroscopic smoothness and uniformity of surface, and microscopic fineness and uniformity of reticulation

2 Cohesiveness and adhesiveness to glass, silver and asbestos when congealed

3 Greasiness of surface, as judged by repulsion and globulation of watery fluids

As a final test, glass tubes of 2 mm internal diameter, coated with the mixture, were tested by aspirating a short column of blood directly from the vein of an animal, and the relative clotting-time of this blood-column was observed and compared with that in a similar tube without coating

The combination finally adopted as the best of twenty-four mixtures tested was

Grubper's filtered paraffin melting point 60 to 62 C (140 to 143 6 F), 56 parts by weight, and pure white petrolatum, 44 parts by weight This mixture has a melting point of 49 to 50 C (120 2 to 122 F)

Experiments to develop the best form of cannula and blood-receptacle were carried out as indicated in the reports of Experiments 1 to 4, inclusive Several glass cylinders having a piston or plunger discharge were abandoned as this type of apparatus was found difficult of control for slow delivery, uncertain in action, required the use of a third hand in effecting the transfer, and was not nearly so delicate as the simple pneumatic discharge assisted by gravity, which was afterward adopted

Experiments with needles and cannulas for introduction into the vein led to many changes before a satisfactory method was evolved It

1 Bernheim B M Jour Am Med Assn 1912, lvii, 1007 Bordet and Genou Ann de l'Inst Past 1903 p 822 Brewer and Legget Surg, Gynec and Obstet 1909 ix, 293 Vincent B Am Jour Dis Child, 1911 i, 376

2 Adler H M The Archives Int Med, 1913, xi 187 Hopkins, A H The Archives Int Med, 1913 xi, 300

was found impossible to coat with paraffin a cylindrical needle and flexible connecting tube in conjunction with a glass container, so as to preserve an intact coating throughout the entire system, and at the same time provide a sufficiently wide channel for effectual aspiration and delivery The wax mixture was therefore, in the next experiments, used for coating the glass containers only, and oil or liquid paraffin was resorted to as a coating for the needles and tubing This makeshift worked with moderate satisfaction on the experimental animal, but is open to at least a theoretic objection on account of the considerable amount of liquid oil which may enter the circulation, and the practical objection that with a flexible connection the whole aspirating apparatus cannot be held and

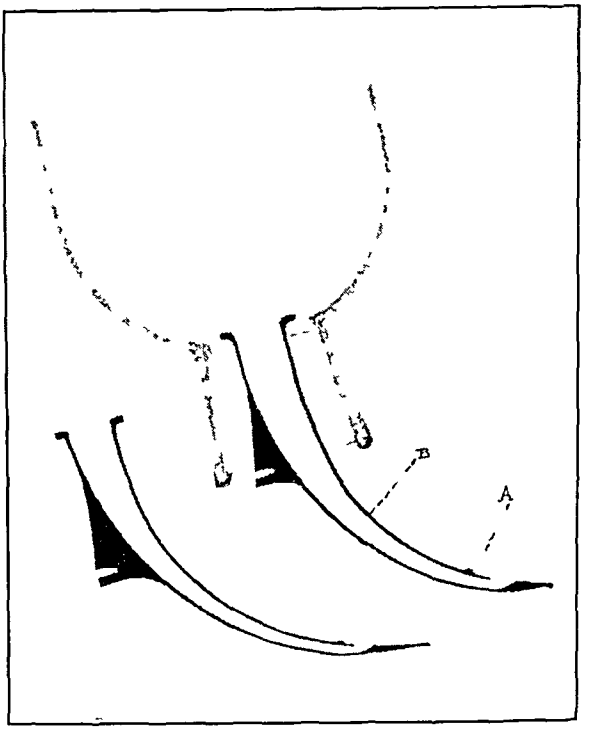


Fig 1 —Longitudinal section of cannula seated in asbestos stopper of pipet

the cannula directed into the vein with one hand, while the other hand of the operator is left free to fix the wall of the vein At this stage of our investigation it became evident that it would be advantageous to devise a special cannula rigidly connected with a glass cylinder, forming a pipet The construction of this instrument was based on several important principles which a diagram will elucidate (Fig 1)

The point of the instrument is conical in shape and is designed to stretch apart the tissues penetrated, while effecting the least possible injury The distal opening of the cannula is placed laterally and the edges of this opening are smoothly rounded The thickening or shoulder

just back of the opening serves as a stop and prevents possible leakage when the cannula is pushed into the vein

It will be seen, by reference to Figure 1 that the cylindrical part of the lumen extends for only a fraction of an inch, and then immediately widens This enlargement, though appearing as a very gradual taper, provides an increase in the circumference proportionate to the increase of the radius, and an increase in the area of the cross-section proportionate to the squares of the radii, so that at the point *B*, which has a diameter double that of point *A*, the circumference of a contained column of fluid is only doubled, but its volume is increased four times This means that at *B* the degree of lateral contact of the column is diminished one-half in relation to the sectional area of the column at that point

The wall of a tube of small diameter, which can be wet by a contained fluid, necessarily determines a certain amount of lateral friction on a

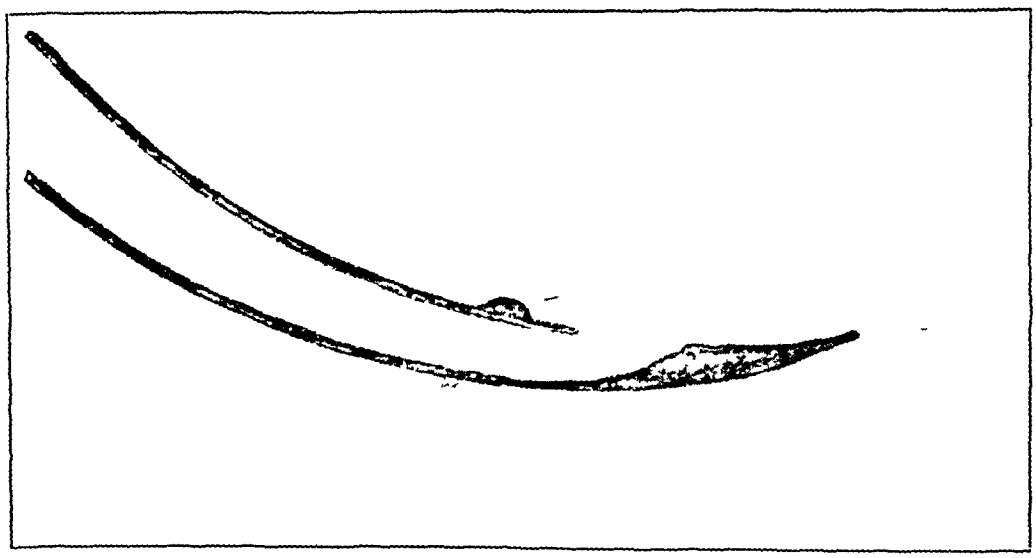


Fig 2—Distal end of cannula showing position in vein Shoulder just back of opening prevents leakage from blood vessel

moving column of that fluid, the peripheral layer of the column adhering so closely to the wall of the tube as to be practically motionless It is obvious that a paraffin coating on the wall of such a tube materially lessens this peripheral friction That friction is also lessened by expanding the caliber of the cannula and reducing its length to a minimum, may be readily deduced from the law of Poiseuille for determining the outflow of fluid from capillary tubes This law may be expressed by the equation

$$Q = \frac{D^4 H}{L}$$

That is to say, the quantity, Q which is discharged in a unit of time is directly proportional to the fourth power of the diameter, D, of the

tube, and to the height of the pressure, H, and indirectly proportional to the length of the tube, L It may furthermore be reasonably assumed, and there is experimental evidence to support the assumption,[3] that diminished friction of blood in a foreign system, is associated with a diminished tendency to coagulation

The important feature of the short attachment of the cannula to the neck of the pipet is contrived by means of an air-tight asbestos stopper, enclosing the proximal end of the cannula and making the latter an arm

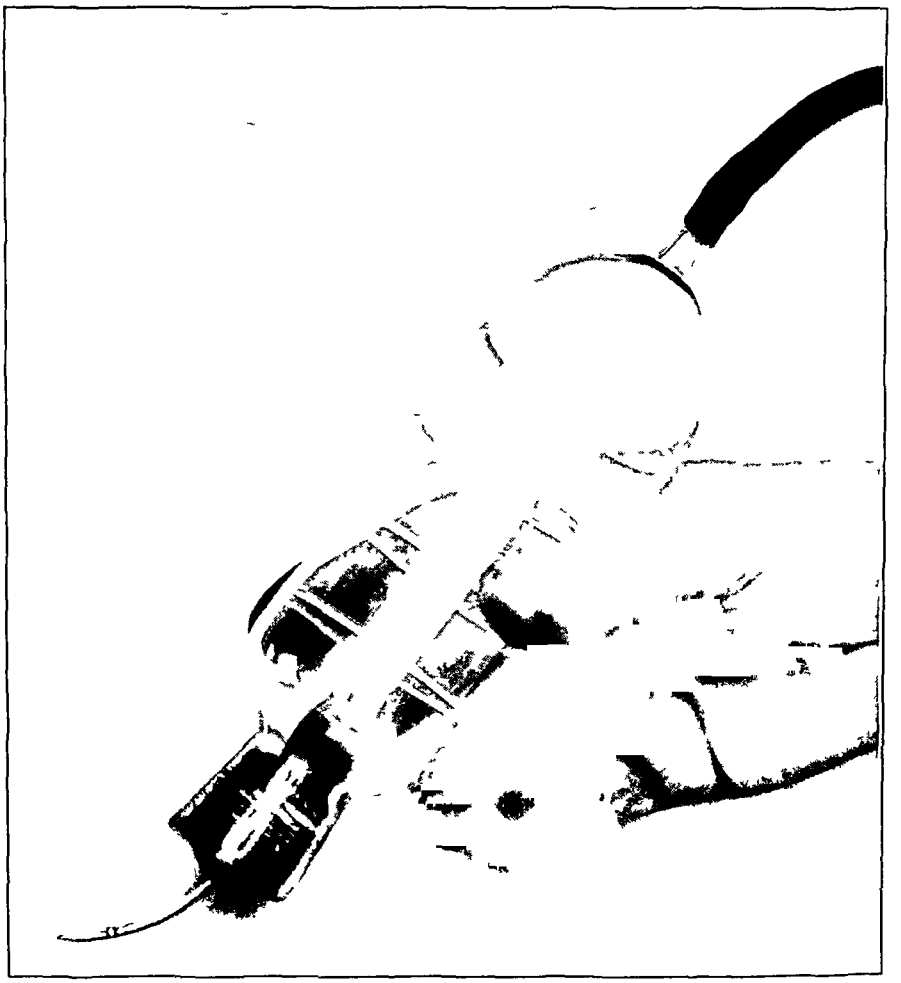


Fig 3—Pipet with cannula in position for insertion in vein

of the pipet, the two members together forming virtually a rigid one-piece apparatus (Fig 3)

METHOD OF PREPARATION AND USE OF APPARATUS

The process of coating the inside of the apparatus with paraffin requires a careful technic to be successful The pipet must be thoroughly cleaned and free from moisture and two coats are applied The first

3 Hurthle K Archiv f d ges Physiol 1900, lxxxii 436 438

coating is done at 68 to 71 C (154 4 to 159 8 F) in a bath containing a quantity of the paraffin mixture sufficient to allow the cylinder to be immersed for its entire length and to a depth of at least half its diameter It is then rotated in the melted wax until uniformly heated Air-bubbles must be avoided in the removal of the cylinder from the bath, and the coating must take place under sterile precautions When the first coating is thoroughly hardened a sterile aspirating tube is attached, and the sterile asbestos stopper and cannula are snugly inserted (Fig 4)

The second coating is applied by aspirating the hot paraffin mixture from the bath, at a temperature of 60 to 61 C (140 to 141 8 F) through the cannula into the cylinder When the inflowing paraffin has reached a level of about 1 cm above the neck of the pipet, it is immediately

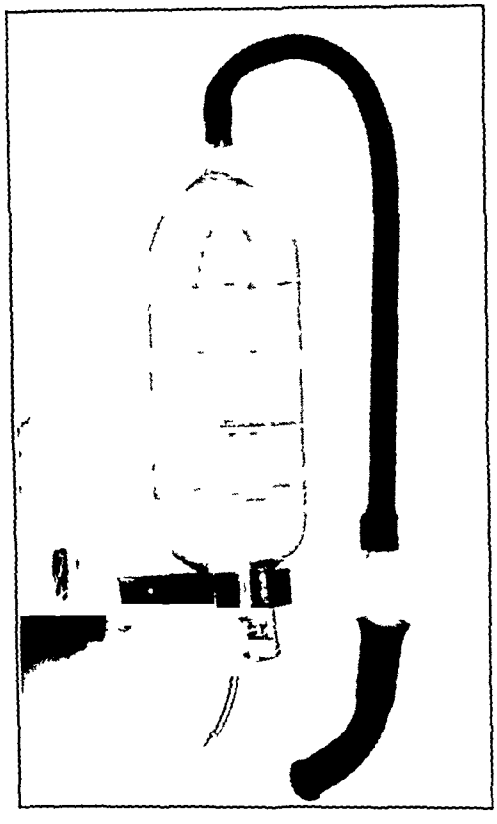


Fig 4 —Pipet and cannula with aspirating tube and mouth-piece attached

expelled The cannula is then lifted from beneath the surface of the bath and its lumen quickly cleared by gentle blowing through the mouth-piece care being taken to avoid any back suction which would result in air-bubbles The cannula is finally protected by covering with a piece of sterile-glass tubing held in place by a strip of adhesive plaster

In warm weather the apparatus should be prepared at least one hour in advance of use so as to allow the paraffin to harden thoroughly If a sharp cannula is used attention must be given not to damage the point when coating the apparatus and in every case its lumen must be smooth and clean before coating

It should be stated here that both sharp and blunt-pointed cannulas have been used The use of the former constitutes the simpler method of penetrating the vein when the size of the blood-vessel admits of free manipulation, but a fine probe point is to be preferred in most cases, especially if the vein is small and the danger of wounding or penetrating the opposite wall is, therefore, increased

METHOD OF TRANSFUSION

The method of transfusing with the blunt-pointed cannula is as follows

Instruments—Knife, scissors, thumb forceps, fixation forceps, one or two mosquito clamps, several self-retaining stylets of different sizes (Fig 5), sutures and needles for closing the skin wound

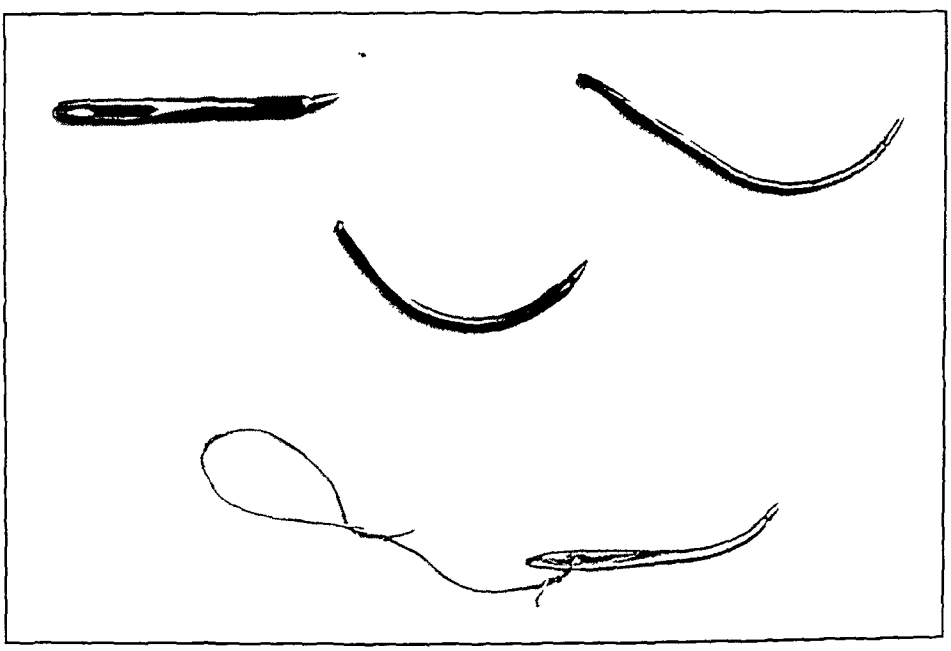


Fig 5—Self retaining stylets used to puncture blood vessel

Apparatus—Several prepared pipets having cannulas of the size likely to be needed for the case at hand, two obturating sleeves with clamps attached, a small tube of sterile liquid paraffin and some sterile petrolatum, a sterile aspirating tube, a cotton air-filter, and a mouth-piece

Operation—The donating and recipient veins are exposed under local anesthesia in the usual manner through incisions about 4 cm in length, and the fascial sheath is cleared away so that a firm hold of the vein-wall can be secured A pipet with a cannula suited to the size of the recipient vein is chosen the aspirating tube with mouth-piece is attached and an obturating sleeve, with clamp attached, is dipped into sterile liquid

paraffin and slipped over the cannula The action of the sleeve is tested to make sure that it will slip easily backward and forward and it is then retracted to a position just proximal to the shoulder of the cannula Suitable stylets are selected, dipped in sterile petrolatum, and inserted through the walls of both veins The direction of penetration should be with the current in the recipient vein and against the current in the donating vein The operator, having taken the mouth-piece and holding the pipet in his right hand, fixes the wall of the donating vein with a forceps in his left hand immediately beyond the point where the stylet has just been introduced, and exerts slight traction upward The stylet is then quickly removed, by means of its attached loop, with a finger of the right hand the traction on the vein controlling the outflow of blood, and the cannula is then pushed through the small hole which has been punctured by the stylet The arm of the donor is now constricted above, and the blood is drawn into the pipet by aspiration When enough blood has been obtained the cannula is withdrawn from the vein with one hand, while with the other hand the obturating sleeve is pushed to its distal position covering the mouth of the cannula a moment after it emerges from the vein A little blood is purposely allowed to flow from the cannula at this juncture to remove any thromboplastic substance which may have been collected from the broken surface of the vessel-wall at the moment of withdrawal The duties of an assistant, who may be the donor himself, are to remove the constriction of the arm when the blood has been obtained and to press a sterile gauze compress over the wound until it is ready for suture Introduction of the blood into the vein of the recipient is accomplished in precisely the same way as just described for its withdrawal, except that the fourth or fifth finger of the right hand of the operator is used to retract the obturating sleeve at the moment of entering the vein

The capacity of the pipet which has been found most useful is about 225 c c It is probably inadvisable to take more than 200 c c of blood at one time A second pipet can be used for a second transfusion if desirable Not more than twelve minutes should elapse in making a single transfusion with safety One hundred and fifty c c should be obtained in from two to five minutes, depending on the abundance of blood-flow in the donor's arm, and this should be injected in from four to six minutes There is probably no danger of overdistending the right auricle if delivery is effected no faster than 50 c c per minute for an adult, and 20 to 25 c c per minute for a child

In conclusion we present, in chronologic order and as briefly as the details will permit, a report of our experiments in transfusing animals with this apparatus, together with our clinical application of the method in three cases

EXPERIMENTAL AND CLINICAL REPORTS

EXPERIMENT 1.—Feb. 12, 1913. Dog 1, male, 5.81 kg. Ether anesthesia. Femoral and external jugular veins exposed.

Apparatus: glass cylinders with asbestos packed piston plungers, two openings at opposite ends of cylinder, one for aspiration and one for delivery. Cylinders previously coated with the plunger in position for delivery. Paraffin mixture 2. Delivery tube of rubber, 5 cm. in length, coupling the glass cylinder to an ordinary hollow needle of medium size (1.16 mm. external diameter). Delivery tube and needle not coated.

Aspiration of blood accomplished by suction with rubber bulb connected by tubing to proximal opening of cylinder provided for the purpose. When cylinder was filled with blood this opening was shut off and delivery effected by advancing the plunger. The tubing and needle were tried at first dry and then with an internal coating of oil. The blood clotted promptly in the tube and needle in the first instance, and less promptly in the tube and needle which had received the coating of oil. A much more satisfactory delivery was effected by the use of a tapered cannula with oil coating. The blood remained fluid in the paraffin lined glass cylinders from nine to eleven minutes, the temperature of the room being about 20 C. (68 F.). The action of the plunger was found to be uncertain and difficult to control. The dog was killed at the end of the experiment.

EXPERIMENT 2.—Feb. 14, 1913. Dog 2, female, 6.34 kg. Ether anesthesia. Both femoral and external jugular veins exposed.

Apparatus: glass cylinder, about 80 c.c. capacity, previously coated with paraffin mixture No. 7, having two openings at opposite ends, one for pneumatic aspiration and expulsion of the blood and the other for connection as in last experiment. A specially modified tapered cannula and large size (1.62 mm. external diameter) cylindrical needles were employed. Cannula, needles and connecting tubes were coated with oil.

First trial (tapered cannula): 60 c.c. of blood taken from jugular and without difficulty discharged into femoral vein. The small amount of blood remaining in cylinder clotted in twelve minutes thirty seconds.

Second trial (cylindrical needle): transfusion not effected owing to clotting in needle.

Third trial (tapered cannula): 40 c.c. taken from femoral and without difficulty discharged into jugular. The small amount of blood received in cylinder clotted in approximately fifteen minutes. The dog was killed at the end of the experiment and the veins were dissected out and opened. No evidence of clotting was in the veins.

EXPERIMENT 3.—Feb. 15, 1913. Dog 3, male, 9.9 kg. Ether anesthesia. Aseptic precautions. Right femoral and right jugular veins exposed.

Apparatus: glass cylinder communicating directly through stopper with a specially curved and tapered cannula. The entire apparatus provided with an intact coating of paraffin mixture 9. The result of the preliminary test of the coagulation time of the dog's blood (Bier-Brooks method) was that at 37.5 C. (99.5 F.) at the end of two minutes there was a slight fibrin formation, at two minutes forty seconds a slight fibrin formation, at four minutes a complete clot. About 48 c.c. of blood were aspirated from the jugular vein by means of a rubber bulb aspirating tube; was clamped, the cannula withdrawn and the hole in the vein clamped with mosquito clamp and subsequently tied with fine catgut. There was considerable dripping of blood from the cannula which obscured the field of operation and required sponging with salt solution. The cannula was introduced into the exposed femoral vein in the same manner and about 45 c.c. of blood was discharged and the puncture hole tied off as before. The time of entrance of the cannula into the first vein to the withdrawal from the second

vein was a little more than four minutes Most of the blood reserved in the pipet was allowed to run out six minutes five seconds after withdrawal from the first vein, and a four-loop preparation of this was taken and transferred to the chamber of a Biffi-Brooks coagulometer with observation as follows

Temp 35 C (95 F), forty seconds, no clotting

Temp 34 6 C (94 2 F), one minute forty seconds, no clotting

Temp 33 2 C (91 7 F), four minutes thirty-five seconds, fairly firm clot

The skin wounds were closed, collodion dressing applied and the dog allowed to come from under the anesthetic The small amount of blood reserved in the pipet was examined at thirty-five minutes after withdrawal from the vein That portion remaining in the cannula and in the cylinder adjacent to it was found clotted, but the larger portion which had flowed back in the cylinder as it lay in the horizontal position was still fluid A loop of this was removed to the coagulometer and found to dissipate completely when plunged into water The dog appeared perfectly normal after the operation and showed no subsequent ill effects

Clinical Trial, Case 1.—Feb 16, 1913 E B, a girl aged 7½, was admitted to the hospital Jan 24, 1913, with scarlet fever, in the eighth day of her illness Temperature 103 F, pulse 138, respiration 24, heart, lungs, abdominal organs and urine were negative on admission The patient appeared to be making a good recovery until February 6, when she had a chill and a sudden rise of temperature to 105 8 F, and several days later developed signs of a nephritis By February 12 she had a fully developed purpuric rash with scant bloody urine and joint pains February 14 an uncontrollable oozing hemorrhage started from the nose, the condition of the patient became rapidly worse and a systolic heart-murmur developed Horse serum, 10 c c, was given subcutaneously February 15 the epistaxis continued, there was evidence of cardiac dilatation on percussion, vomiting of blood recurred at intervals, the hemorrhagic rash was very marked and widely distributed Horse serum plugs were placed in the nostrils and 20 c c horse-serum given subcutaneously February 16 the patient vomited bloody fluid continually, retained no food or medication by mouth, the temperature was 98 4 F, pulse 160 and hardly perceptible at the wrist, respiration 26, a tarry stool was passed at 4 p m, the condition was evidently moribund The father was summoned and offered to serve as donor for a transfusion Apparatus similar to that in Experiment 3 was used, and 65 c c of blood was removed from the donor's median basilic vein and injected into the median cephalic vein of the patient The transfer took place in approximately three and one half minutes No difficulty of any kind was experienced except for the dripping of blood from the cannula at the moment of entering the recipient vein, which required sponging The small amount of blood remaining in the pipet did not clot until twenty-one minutes after withdrawal from the vein The patient's pulse and mental condition seemed slightly better shortly after the transfusion, but death occurred early on the morning of the following day

A condensed report of the macroscopic findings at the autopsy, performed by Dr George P Biggs fourteen hours after death, follows Heart distinct dilatation of the left ventricle, petechial hemorrhages of the endocardium and hemorrhagic areas in the papillary muscles on cross section Kidneys considerably enlarged capsules not adherent, surface smooth, pyramids and cortex both greatly swollen, cortex light in color and markings indistinct Suprarenals rather large, left containing a small area of softening in the upper portion, the right a small area of softening in the lower portion, no definite hemorrhages Ulcerative lesion of the left tonsil and base of the tongue with erosion of blood-vessels Multiple hemorrhagic areas of the lungs and spleen Petechial areas widely distributed over skin, mucous membranes and serous membranes

Experiment 4.—March 11, 1913 Dog 3 (the dog used in Experiment 3) Ether anesthesia Right jugular and right femoral veins exposed

Apparatus A new pipet, similar to that used in last experiment, but graduated and arranged for mouth aspiration with sterile cotton air-filter A sharp tapered cannula was used as in Experiment 3 but was cemented into an asbestos stopper This was made by winding very thin asbestos tape, 1 inch wide, about the shank of the cannula as a core The fiber of the asbestos was bound centrally and marginally by impregnation with a solution of sodium silicate, and medium size asbestos twine was used as an outer binding The apparatus was coated with paraffin mixture 10

One hundred c c of blood were transfused from the jugular vein to the femoral The transfer was easily effected except for dripping blood when entering the second vein The time of operation was approximately six minutes The same amount of blood was removed from the femoral and partially discharged into the jugular, some difficulty was experienced in entering the vein due to the sharp cannula, insufficient exposure of the blood-vessel and obscuring of the field by dripping blood The dog was killed at the end of the experiment The same veins were used as in Experiment 3 with this dog, and necropsy showed them to be entirely patent and with no evidence of thrombosis

CLINICAL TRIAL, CASE 2.—March 16, 1913 G S, a girl aged 10, was admitted to the hospital March 2, 1913, with severe scarlet fever of a septic type There was a profuse nasal discharge and pronounced angina A throat culture was negative for Klebs-Loeffler bacilli March 5 130 c c. of antistreptococcus serum were given without effect The patient became rapidly worse, and on March 6, the condition being desperate, it was planned to make a transfusion with the blood of a convalescent scarlet-fever patient who volunteered for the purpose One hundred and fifty c c of blood were quickly and without difficulty removed from the median basilic vein of the donor, and an attempt was made to transfuse this into the median basilic vein of the patient This latter blood-vessel was insufficiently exposed to view and took a turn just above the upper extremity of the skin incision Dripping of blood from the cannula further interfered with the accurate introduction of the cannula, which was a sharp one, and the vein was completely penetrated several times before its canal was finally entered About 20 c c was eventually delivered, and although the blood remaining in the cannula appeared perfectly fluid, the length of time which had elapsed, about fifteen minutes, made it seem somewhat risky to continue the procedure beyond that time A further injection of blood was therefore abandoned The blood clotted in the cannula at about nineteen minutes, and began to clot in the cylinder at twenty-two minutes

There was, of course, no effect to be anticipated from so small an amount of blood, and the operation had to be reckoned a failure The patient died March 7 and no necropsy was allowed The apparatus used in this operation was the same as noted under Experiment 4

EXPERIMENT 5.—March 17, 1913 *To test action of cannula sleeve as an improvement in technic*.—Dog 4, male, 11 65 kg Ether anesthesia Aseptic precautions Both femoral veins exposed

Apparatus a 200 c c graduated pipet with a special tapered cannula Paraffin coating, mixture 10 An elastic cannula sleeve was contrived by using a piece of fine rubber tubing 1 cm in length This was held by clamping it longitudinally in a Carrel serrated clamp, and it was dipped in sterile liquid paraffin prior to use

The right femoral vein was entered with the sleeve in position proximal to the shoulder of the cannula, and 140 c c of blood was aspirated as fast as could be obtained from the vein As the cannula was removed the sleeve was pushed into a position distal to the shoulder thus covering the opening of the cannula No dripping ensued The cannula was now entered as far as the sleeve through the wall of the recipient vein the sleeve was retracted proximally to the shoulder and the cannula pushed on into the vein up to the shoulder, allowing

the escape of only a few drops of blood One hundred and thirty-five c c of blood were allowed to run from the pipet by gravity assisted by gentle blowing The time of transfer was four minutes fifteen seconds No ligatures were necessary, the skin was sutured and a collodion dressing applied The dog was allowed to come from under the anesthetic and made a good recovery

Observations on the coagulation time of the blood in this experiment will be found in Table 1

TABLE 1—Blood Coagulation Time Experiment 5

	Temp C	Time	Occurrence of Clot
Preliminary coagulation time	37	2′ 30″	None
	37	2′ 45″	Firm
Blood dripping from cannula after withdrawal from vein 7′ 35″	37	20″	None
	37	1′ 00″	Partial
	37	1′ 10″	Firm
13′ 45″	44	30″	None
	44	1′ 15″	Partial
	44	1′ 30″	Firm
18′ 35″	43	1′ 00″	Very slight
	43	1′ 45″	Partial
	43	2′ 15″	Firm
21′	42	1′ 00″	Partial
	42	1′ 30″	Partial
	42	2′ 00″	Firm
25′ and 30′	Blood still dripped from cannula, coagulation time not taken.		
35′	Blood did not drip from cannula, but could be easily blown through		

It should be noted in regard to these determinations, which were by the Biffi-Brooks method that the loopfuls of blood obtained for observation varied considerably in volume, owing to the fact that the blood was of necessity allowed to drip from the cannula on the platinum loops, instead of being collected in the usual manner by passing the loops over the blood This, of course, invalidates the accuracy of this method for determining the clotting time of blood dripping from the cannula The application of the cannula sleeve in this operation was found very satisfactory and effective

Experiment 6—April 1, 1913 *Illustrating error of technic in coating*—Dog 5 male, 13 6 kg Ether anesthesia Aseptic precautions Both femoral veins exposed

Apparatus a 200 c c pipet and a 150 c c pipet provided with tapered cannulas and cannula sleeves as in Experiment 5, and coated with paraffin mixture 10 After the second coating both cannulas were briefly heated over the free flame of a Bunsen burner to reduce to a minimum the amount of wax within their lumen (This was later recognized as an error of technic)

A preliminary test of the dog's blood gave a coagulation time of one minute thirty five seconds at 37 C (98 6 F) by the method of Dale and Laidlaw This method was chosen as being best suited to the conditions of the investigations,

and was especially satisfactory on account of the sharply defined end point of the determination

With the first pipet about 60 c c of blood were removed from the right femoral vein and injected into the left femoral vein Clotting occurred shortly after the introduction of the cannula into the recipient vein The clot was removed, and at eight minutes the injection was resumed, and all but a small remainder was delivered without further clotting At nine minutes the coagulation time of the blood reserved in the pipet was one minute twenty five seconds at 37 C (Dale-Laidlaw)

With the second pipet a second transfusion was attempted from the right femoral to the left femoral veins, and clotting again occurred in the cannula shortly after its introduction into the recipient vein This clot was removed, and about 80 c c of blood were injected into the vein at four minutes, the remainder being reserved in the pipet for observation At eight minutes the coagulation time of blood dripping from the mouth of the canula was found to be one minute twenty-five seconds at 37 C (98 6 F) Between nine and ten minutes it was found that the blood had again clotted in the cannula, but that in the cylinder remained fluid for more than thirty minutes Some of this was removed from the cylinder at thirty minutes and tested for coagulation time It was found to be in two minutes ten seconds at 30 C (86 F) [4] The wounds were sutured, dressing applied and the dog allowed to come from under the anesthetic Good recovery

EXPERIMENT 7.—April 3, 1913 *To test the correctness of conclusions from Experiment 6 as to technic of coating*.—Dog 5, male, 13 6 kg (dog used in experiment 6) Ether anesthesia Aseptic precautions Both external jugular veins used

Apparatus Two pipets of 120 c c and 200 c c capacity, respectively, provided with tapered cannulas, carefully coated at the proper temperatures and without flaming, paraffin coating 12

The right jugular vein was entered against the current and about 80 c c of blood were removed and transferred to the left jugular vein with the current, 2 to 3 c c being allowed to remain in the pipet The time of transfer was two minutes ten seconds Preliminary coagulation time was one minute thirty seconds at 37 C by the Biffi-Brooks method, one minute twenty seconds by the Dale and Laidlaw method at the same temperature At sixteen minutes after removal from the vein the blood remaining in the pipet was allowed to drip from the cannula and the coagulation time was found to be one minute twelve seconds at 37 C by the Dale Laidlaw method

The left jugular vein was entered with the second pipet, against the current, and 150 c c of blood were removed All but 4 or 5 c c of this was transferred to the right jugular vein The blood was withdrawn from the vein as rapidly as it would flow, the time being one minute forty seconds The heart's action became coincidently very much accelerated, as also the respiration The blood was more slowly introduced into the right jugular vein to avoid overdistention of the right auricle The time was three minutes forty five seconds The amount was 145 c c The total time of transfer was five minutes thirty seconds During the reintroduction of the blood the pulse and respiration fell to about the same rate as before its removal Coagulation time of blood dripping from the cannula after the operation was as follows

At 6 to 7 minutes after withdrawal from vein
1 minute 10 seconds to 1 minute 30 seconds at 37 C by the Biffi Brooks method
1 minute 10 seconds at 37 C by the Dale-Laidlaw method
At 18 minutes the coagulation time was 40 seconds at 37 C by the Dale Laidlaw method

4 Corrected for temperature according to Burker's curve for human blood[3] this would be one minute forty four seconds at 37 C

At 25 minutes the blood had clotted in the cannula, but was still fluid in the cylinder

Observation of the blood remaining in the pipet was not continued after twenty five minutes The dog was killed at the end of the experiment Necropsy showed a skin infection of wounds in the groins from former operation The femoral veins used in the previous transfusion were patent and contained fluid blood

EXPERIMENT 8—April 7, 1913 *To test further the comparative effects of proper and improper method of second coating*—Dog 6, male, 11 kg Ether anesthesia

Apparatus same as used in the last experiment Two pipets used Pipet 1 received careful attention in its second coating, especially as to proper temperature (60 to 61 C, 140 to 141 8 F) Pipet 2 received its second coating improperly as in Experiment 6, the cannula being gently heated over a free flame to allow the excess of paraffin to be blown out

Both femoral arteries and external jugular veins were exposed Using Pipet 1, 200 c c of blood were taken from left femoral artery in one minute twenty seconds, the artery was clamped and one minute later the cannula was introduced into the left jugular vein All but 8 c c of the blood was injected, time, three minutes forty seconds Total time of transfer six minutes forty-five seconds Preliminary clotting time of dog's freshly shed blood was one minute twenty seconds at 37 C by the Dale Laidlaw method At thirty-four minutes forty-five seconds the blood received in the pipet for observation dripped from the cannula when the sleeve was retracted, and its coagulation time at 35 6 C was forty seconds (Dale Laidlaw) At forty-two minutes forty-five seconds blood still dripped from the cannula when the sleeve was retracted, and its coagulation time was 30 seconds at 35 to 36 C (95 to 96 8 F) by the Dale-Laidlaw method At forty four minutes forty-five seconds the blood had clotted in the cannula, but was still fluid in the cylinder, and remained so until sixty minutes forty-five seconds, when it had just started to clot

With Pipet 2, 215 c c of blood were taken from the right femoral artery in one minute fifteen seconds, and the greater part of this was transferred to the right jugular vein The total time was five minutes forty-five seconds At ten minutes the blood had clotted in the lumen of the cannula The clot was removed at fourteen minutes forty-five seconds, and the blood which then dripped away clotted in twenty seconds at 37 C (Dale-Laidlaw) The blood again clotted in the cannula immediately, but remained fluid in the cylinder until twenty-two minutes, when it began to clot The dog was killed at the end of the experiment

EXPERIMENT 9—April 16, 1913 *Illustrating effect of trauma by stretching and abrasion of intima*—Dog 7, male, 6 6 kg Ether anesthesia Aseptic precautions The same apparatus as used in Experiment 8 Preliminary coagulation time at 37 C, one minute twelve seconds by the Dale-Laidlaw method Right femoral and saphenous veins exposed A part of the latter was selected for entry which appeared about half the diameter of the cannula This was, nevertheless, easily entered by stretching and about 80 c c of blood were slowly aspirated The slow withdrawal of blood was due to the small size of the vessel and the pronounced valve action of the stretched wall against the mouth of the cannula The cannula was removed from the saphenous vein at five minutes twenty seconds, and was then introduced into the femoral vein No blood would pass, and the cannula was immediately withdrawn and found to contain a long clot The clot was removed with a small forceps and the cannula again introduced into the femoral vein at nine minutes thirty seconds after entry into first vein Half the amount of blood in the pipet, about 40 c c, was then injected and at ten minutes ten seconds a second clot formation occurred which blocked the cannula This was removed with difficulty, and no attempt was made to inject the blood remaining in the pipet The vein punctures were ligated with fine catgut and the wound

closed in the usual manner with a collodion dressing The dog was allowed to come from under the anesthetic and made a good recovery

At twelve minutes after first withdrawal from the vein the blood remaining in the pipet showed a single string of fibrin, and the blood in the cannula had again clotted At twenty-five minutes there were several long strings of fibrin apparent on rotating the pipet, but the rest of the blood was still fluid At thirty-four minutes a solid clot had formed

EXPERIMENT 10.—April 22, 1913 *To confirm the conclusions from last experiment*.—Dog 7 (dog used in Experiment 9) Ether anesthesia

Apparatus is that used in Experiment 9 Two pipets with sharp pointed tapered cannulas Paraffin mixture 24

Preliminary clotting time of dog's blood forty six seconds at 37 C (Dale Laidlaw) Left femoral and internal saphenous veins exposed, also both external jugulars

With Pipet 1 having a larger tapered cannula, about 160 c c were taken from the right external jugular, against the current, in two minutes ten seconds, and about one-half of this was injected, against the current, into the left external jugular in one minute thirty seconds The total time was four minutes No difficulty was experienced The blood dripping from the cannula was tested as follows

At 9 minutes 30 seconds the coagulation time was 20 to 25 seconds at 37 C

At 34 minutes the coagulation time was 1 minute 2 seconds at 34 C (temperature correction negligible)

At thirty-four minutes there was some fibrin formation apparent in the blood in the cylinder, although the bulk of it was perfectly fluid At forty minutes the blood in the cannula and cylinder had clotted firmly With Pipet 2, which was fitted with the smaller tapered cannula, the left internal saphenous vein was entered This vessel was much smaller in its normal diameter than the cannula, and the conditions of Experiment 9 were closely simulated, that is, the distention and flapping of the vein-wall against the mouth of the cannula Aspiration was very slow, about 50 c c of blood being obtained in eight minutes fifteen seconds A very small portion of this blood (about 10 c c) was injected into the left jugular, and at nine minutes thirty seconds the flow was stopped by a clot in the cannula There was also observed at this time slight evidence of fibrin formation in the fluid blood remaining in the cylinder At nineteen minutes thirty seconds the blood in the cylinder was still fluid, but with more strings of fibrin At thirty-two minutes the blood was still fluid, but there was much fibrin formation Observation was not continued further The dog was killed at the end of the experiment

EXPERIMENT 11.—April 28, 1913 *A variation of the last two experiments to determine the effect of making the recipient vein the traumatized vessel*.—Dog 8, male, 9 5 kg Ether anesthesia Apparatus, two pipets as used in Experiment 10 Paraffin coating 24 Preliminary coagulation time of dog's blood one minute seventeen seconds at 39 C (102 2 F) by the Dale-Laidlaw method Right femoral, left internal saphenous and both external jugular veins were exposed

Pipet 1 was introduced into the right jugular vein and 220 c c of blood withdrawn in one minute forty-five seconds One minute elapsed in transferring to the right femoral, and 200 c c were injected into this vessel in one minute forty five seconds, making the total time of transfusion four minutes thirty seconds Both tributary and recipient veins were of ample size and no hindrance was met Observations on coagulation of the 20 c c of blood remaining in the pipet were as follows

At 21 minutes 22 seconds, flowed from cannula Fluid in cylinder, no fibrin Coagulation time 1 minute 55 seconds, 37 C

At 25 minutes, clotted in cannula, fluid in cylinder, with some fibrin formation

At 35 minutes, clotted in canula, fluid in cylinder, with much fibrin formation

At 38 minutes clotted in cannula, firm clot in cylinder

Pipet 2 was introduced into the left jugular vein and 200 c c of blood withdrawn in three minutes forty-five seconds The internal saphenous vein selected for the recipient vessel was very small and was much traumatized and distended in effecting an entrance When the cannula was in place it was found that the blood could not be freely injected, even with considerable pressure At eight minutes fifteen seconds the instrument was withdrawn and another unsuccessful attempt was made to inject the same vein at a more proximal point This also permitted too slow a delivery on account of the small size of the vessel, and at nineteen minutes the vein was penetrated at its junction with the femoral, where there was a free opportunity for discharge and about 150 c c of blood were then delivered from the pipet At twenty three minutes thirty seconds the blood was found to have clotted in the cannula and there was considerable fibrin formation in the cylinder At twenty-five minutes more fibrin appeared and at thirty three minutes a firm clot existed The dog was killed at the end of the experiment Necropsy showed no blood clots in the right heart or in the pulmonary arteries

Clinical Trial Case 3—April 29 1913 E M, a boy, aged 11, had osteomyelitis of the femur with general staphylococcus septicemia, which had run a chronic course since his admission to the hospital and an operation six weeks previously Temperature reached 102 to 103 F daily Hemoglobin 30 per cent *Staphylococcus aureus* was obtained from the blood culture An uncle had been immunized with vaccines derived from the infecting organism of the patient for the purpose of transfusing the staphylococcus immune blood The donor's and recipient's median basilic veins were exposed under local anesthesia and transfusion carried out with apparatus as used in Experiment 11 One hundred and fifty c c of blood were removed in two minutes fifty seconds, and 100 c c of the amount were injected in nine minutes The recipient's vein was unusually small The total time was thirteen minutes fifty seconds The blood remaining in the pipet, reserving a very little for observation, was allowed to flow on sterile gauze for local application to the suppurating sinuses At twenty minutes there was evidence of clot formation in the cannula and a few shreds of fibrin could be seen on agitating the blood remaining in the cylinder, at twenty six minutes there was a fairly firm clot in both cylinder and cannula May 9, ten days later, the patient's hemoglobin was 80 per cent, temperature practically normal and local condition improved

DISCUSSION OF RESULTS

In the preliminary part of the experimental work, that is, up to Experiment 5, the main requirements of an efficient apparatus were realized These essential features are (*a*) rigid pipet, arranged for pneumatic aspiration and discharge, terminating in a tapered cannula with a smoothly walled channel, the point of the cannula being conical in shape and having an opening of sufficient size to provide a rapid flow of blood, (*b*) a smooth unbroken coating of a suitable paraffin mixture throughout the apparatus, (*c*) a means of closing the opening of the cannula at will, supplied by the obturating sleeve as described in Experiment 5

In the course of further experimentation it was found that there were several errors of technic which materially affected the success of the operation, and which by proper attention could be avoided The first of these errors was in the matter of coating the apparatus In Experi-

ment 6, the clotting in the cannula was a bad feature and was evidently due to heating the cannula over the free flame after the second coating In Experiments 7 and 8 this question was further tested and the conclusion was reached that a fairly low temperature at the second coating is essential in order to insure an intact wall of paraffin in the channel of the cannula, without which there is a tendency to clot formation in the cannula and also, to some extent, in the cylinder above

TABLE 2.—BLOOD COAGULATION TIME IN CLINICAL CASES 1 TO 3, AND EXPERIMENTS 5, 7, 8, 10 AND 11*

Case and Experiment No	Time of Occurrence of Clot Formation in Cannula	Time of Occurrence of Clot Formation in Cylinder
Clinical Case 1	15 to 20′	21′
Clinical Case 2	19′	22′
Dog 4, Exp 5	35′	After 35′
Dog 5, Exp 7 (Transf B)	25′	Still fluid at 25′
Dog 6, Exp 8 (Transf A)	44′, 45″	60′, 45″
Dog 7, Exp 10 (Transf A)	34 to 40′	34 to 40′
Dog 8, Exp 11 (Transf A)	25′	38′
(Transf B)	23′, 30″	33′
Clinical Case 3	20′	26′

* In Table 2 are placed the results of observations on the periods of time which elapsed before coagulation of the blood in the cannula and in the cylinder in those instances in which the conditions of the experiment can be considered normal, that is to say, in which no special factors were introduced to influence an early clot formation

It was also demonstrated in Experiment 8 that arterial blood can be very quickly obtained by this method Whether this would usually necessitate clamping and tying of the artery, or whether the rent in the arterial wall can be repaired so as to preserve a functionating vessel as with a vein, we are, so far as our experience goes, unable to say, but we believe that the use of arterial blood may prove to be a valuable application of the method in clinical practice

Another important error, illustrated in Experiments 9, 10 and 11, was the mistake of inviting clot formation by allowing an undue access of thromboplastic substance from stretched and broken tissue In Experiment 9 the small donating vein was considerably stretched, and

its intima was probably further damaged by flapping against the edges of the mouth of the cannula, with the result of an early and repeated clot formation In Experiment 10 the blood from the traumatized vessel showed evidence of fibrin formation in the pipet at nine minutes thirty seconds as against thirty-four minutes in the control operation where no trauma was induced In Experiment 11 the traumatizing of the recipient vein, on the other hand though doubtless leading to a liberation of thromboplastin, did not produce the early clotting effect in the cannula which was observed when the donating vein was the injured vessel The most probable explanation of this observation is that the current of outflowing blood from the cannula carried away from the instrument into the circulation any thromboplastin which may have been formed at the site of puncture

It is noticeable that there is a considerable range in the period of fluidity the variation being from nineteen to forty-five minutes in the cannula and from twenty-one to sixty minutes in the cylinder The cause of this variation is a matter of speculation, but the most plausible explanation would seem to be that there are differences in the degree of manipulation and trauma incidental to each case and that these differences resulted in a variation of the amount of thromboplastin set free Among other possible factors it may be surmised that the vascular tissues of some animals are more friable than others and that the thromboplastic substance in the tissue lymph of some individuals is more abundant or more active than in others It should also be noted that in Dog 6, Experiment 8, Transfusion V the comparatively long persistence of fluidity may have been due to a set of factors peculiar to this experiment, namely, that the vessel punctured was an artery, the blood arterial, and the speed with which it was obtained was very much greater than in the other experiments

CONCLUSIONS

Generally speaking, a careful technic in the method of transfusion here described is of paramount importance, and should be based on two main principles The first is that there shall be no lapse of the paraffin coating to act as a starting-point for the arrest and disintegration of the formed blood elements, or in whatever other way such a moistenable point may act in initiating a formation of thromboplastin The second is that there shall be the least possible injury to the tissues of the donating vessel which are exposed directly to the transferred blood, lest there be, through such injury, an admixture of thromboplastin liberated with the tissue juices The necessity for care in guarding against these two liabilities constitutes the one factor which militates against the simplicity

and wide usefulness of the method, and it must therefore be considered a disadvantage The practical advantages may be summed up as follows

1 An apparatus has been contrived which is compact, which may be easily and precisely manipulated with one hand, and which may be prepared in readiness for immediate use in an emergency

2 The operation may be easily performed by a single operator without skilled assistance, and the requisite facility in surgical technic is possessed by anyone who is able to perform an ordinary intravenous saline infusion

3 The arrangement for pneumatic aspiration and discharge of the blood affords a delicate and convenient means of control

4 The use of the special stylets for puncturing the exposed vein-wall insures an entrance of the cannula on the first trial, and by this method only a small skin incision is necessary The vessels used are neither divided nor tied off and the puncture hole is very small, so that subsequent function is unimpaired

5 The flow of blood in the donor's and the recipient's veins is not interrupted at the site of operation, and the danger of clot formation in clamped vessels is therefore not encountered

6 The amount of blood transfused and the rate of flow can be accurately observed

7 The donor and recipient may be separated by any convenient distance, and the risks of tearing apart are eliminated

8 The materials of the apparatus are glass, silver and asbestos, all of which can be sterilized by dry heat in an autoclave or in an ordinary oven

In a more extensive test of this method of transfusion under varying clinical conditions, modifications of technic may be advisable As the aim is to perfect a method of wide usefulness to the general practitioner, there are certain improvements still to be sought in the way of greater simplicity and facility For instance, it would be desirable to work out a means of using the apparatus safely without a double paraffin coating, or possibly to dispense with a complete coating of the cylinder by a preliminary flushing of the whole apparatus with a solution of hirudin in normal salt solution An improvement in technic would be realized if a simple device were provided for covering the mouth of the cannula during its passage into the donor's blood-vessel, so as to afford protection from contact with broken tissue at the point of puncture From the indications of work already done toward this end, it is not improbable that some such improvements can be worked out Meanwhile we believe that the results of our experiments have been of sufficient interest to

report, and that the method so far developed should have a more extensive clinical trial to determine its usefulness

We wish here to express our thanks to Professor Frederic S Lee for his kind interest in our work, and for valuable suggestions in the preparation of this paper

175 East Seventy First Street

REFERENCES

In addition to the numbered references the following are referred to in the text

Addis, T Jour Infect Dis, 1912, x, 200

Brodie, T G Jour Physiol, 1900 1901, xxvi 48

Burker K Arch f d ges Physiol, 1912 cxix 318

Cooley and Vaughan Jour Am Med Assn, 1913 lx, 435

Crile, G W Hemorrhage and Transfusion, D Appleton & Co, 1909

Curtis and David Jour Am Med Assn, 1911, lvi, 35, 1911, lvii, 1153, Surg, Gynec and Obstet, 1912, xv, 476

Dale and Laidlaw Jour Path and Bacteriol, 1912 xvi 351

Duke, W W Jour Am Med Assn, 1910, lv, 1165, Jour Exper Med, 1911, xiv 265, The Archives Int Med 1913, xi, 100

Edelberg, M Arch f exper Path u Pharmakol, 1880, xii 283

Esmarch, F Handbuch der Kriegschirurgischen Technic, 1877, p 165, Surgeon s Handbook, 1888 Eng transl 3d Ger ed, p 213

Esmarch and Kowalzig Surgical Technic, 1901, Eng transl by Grau & Sullivan, p 278

Falta W, and Fleming, G B München med Wchnschr, 1911, lviii 2049

Fuld E Zentralbl f Physiol, 1903, xvii, 529

Genou, O Ann de l'Inst Past 1901, xv, 232

Howell, W T Am Jour Physiol 1910, xxvi, 453, 1911, xxix 187, 1912, xxxi, 1

Hurter, J Beihefte z med Klinik, 1911, No 12

Kohler, A Inaug Diss, Dorpat, 1877

Lindeman, E Rep Ped Sect, New York Acad Med, April 10, 1913

Moldovan, J Deutsch med Wchnschr, 1910 xxxvi, 2122

Morawitz, P Ergebn d Physiol, 1905, iv, 307, München med Wchnschr, 1907, liv, 767

Moritz, F München med Wchnschr, 1911, lviii, p 395

O'Connor, J M München med Wchnschr, 1911, lviii, 1139, Arch f exper Path u Pharmakol, 1912 lxvii, 105

Pike, Guthrie and Stewart Jour Exper Med, 1908, x, 371

Poiseuille Ann de Chimie et de Physique, 1813, Series 3, viii 59

Risley and Irving Boston Med and Surg Jour, 1912, clxvi, 956

Schultz, W Deutsch Arch f klin Med, 1905, lxxxiv, 541

Sollmann, T Am Jour Physiol, 1905, xiii 291

Stevens, L T, and Lee, F S Studies fr Biol Lab Johns Hopkins Univ, 1884, iii, 99

Stewart, H A, and Harvey, S C Jour Exper Med, 1912, xvi, 103

Whipple, G H The Archives Int Med, 1912, ix, 365

Ziemssen, H Verhandl d Kong f inn Med, No 11, Leipsic, 1892, 166, Deutsch Arch f klin Med, 1892, l, 491, München med Wchnschr, 1894, xli, 349, 1895, xlii, 301

THE CONDITION OF THE BLOOD IN HEMOPHILIA THROMBOSIS AND PURPURA *

W H HOWELL, M D
BALTIMORE

During the past two years I have made systematic examinations of the blood in patients suffering from various forms of diseases of the blood, hemorrhagic and thrombotic, with special reference to the variations in the amount of antithrombin and prothrombin The most significant results have been obtained in cases of hemophilia and in so-called idiopathic or spontaneous thrombosis The chief object of the present paper is to report the findings in these two latter conditions

METHODS

The specimens of blood used for examination were obtained in all cases from one of the superficial veins of the arm by means of a graduated syringe (Luer form) The syringe was sterilized previously with boiling water and before inserting the needle into the vein it was filled with sterile salt solution (sodium chlorid 0 9 per cent) by partially filling the syringe and then expelling the solution so as to leave the needle full The object of this precaution was to avoid the bubble or two of air in the needle which otherwise interfered with the accuracy of the reading of the amount of blood taken Usually 8 c c of blood were taken for each experiment and as soon as possible the portion to be examined for antithrombin and prothrombin, namely, 4 c c, was emptied into a centrifugal tube containing 0 5 c c of a 1 per cent solution of sodium oxalate (made up in a 0 9 per cent solution of sodium chlorid) After mixing by inverting the tube this specimen was centrifugalized at a high speed to obtain a clear plasma The plasma, which in human blood is always deeply colored, yellowish green, was pipetted off and examined as follows

Antithrombin.—To determine the relative amount of antithrombin in the oxalated plasma of the patient it was compared always with a similar specimen taken at the same time from a normal person The two plasmas were heated in a water bath slowly to just 60 C At this temperature (53 to 60) the fibrinogen is all precipitated and the thrombin and prothrombin are destroyed, while the antithrombin on the contrary is not affected, or at least is affected as little as possible consistent with the necessity of removing the fibrinogen and thrombin Only at temperatures above 65 C does the antithrombin show a distinct diminution in strength

To test the antithrombin in the heated plasmas they were added in known amounts to varying mixtures of thrombin and fibrinogen The thrombin was prepared in quantity by a method previously described[1] and was kept in dry condition in watch crystals, each crystal containing the same amount For each test

* From the Physiological Laboratory, Johns Hopkins University
* Submitted for publication Nov 24, 1913

1 Howell Am Jour Physiol 1910 xxvi 453 For modification see also same 1913 xxxii 264

the contents of one of these crystals was dissolved in water (4 to 8 c c)[2] For fibrinogen solutions I have not found it convenient to use so called pure fibrinogen prepared by the method of Hammarsten, since it is not possible by this method to obtain solutions of uniform concentration or properties The repeated precipitations of the fibrinogen denatures it in part In place of such solutions I have used the oxalated and dialyzed blood plasma of dogs or cats To get this material a fasting animal was bled into an oxalate solution nine parts of blood to one part of a 1 per cent solution of sodium oxalate made up in sodium chlorid, 0.9 per cent This mixture was centrifugalized the plasma was pipetted off and the excess of oxalate was removed by dialyzing in collodion tubes for twenty four hours against a solution of sodium chlorid 0 9 per cent The plasma thus obtained does not clot spontaneously but coagulates promptly on the addition of a little thrombin A large quantity of this material was prepared from one animal and it was dried in watch crystals 3 to 5 c c to a crystal in a current of cold air from an electrical fan When needed the contents of one or more of these crystals was dissolved in a known amount of a 0 9 per cent solution of sodium chlorid The solution obtained is quite clear In the tests described below these solutions of dried plasma will be spoken of as fibrinogen solutions Ten drops of this solution when mixed with 2 3 4 and 5 drops of the thrombin solution clotted in from two to five minutes To determine the antithrombin in the heated oxalated plasmas described above the following mixtures were made

1 10 drops fibrinogen sol + 1 drop heated plasma + 2 drops thrombin solution
2 10 drops fibrinogen sol + 1 drop heated plasma + 3 drops thrombin solution
3 10 drops fibrinogen sol + 1 drop heated plasma + 4 drops thrombin solution
4 10 drops fibrinogen sol + 1 drop heated plasma + 5 drops thrombin solution

Inasmuch as the effect of antithrombin on thrombin varies greatly with the time allowed for their interaction it is necessary in these experiments to add first the drop of heated plasma to the 2, 3, 4 and 5 drops of thrombin solution and to allow these mixtures to stand for a definite time before adding the solutions of fibrinogen The time interval adopted in all my experiments for this action of the antithrombin and thrombin was fifteen minutes and care was taken to see that this interval was identical within a second or two in all comparative tests At the expiration of the fifteen minutes the fibrinogen solution was added and the time of coagulation of each specimen was observed The mount of antithrombin contained in one drop of heated normal plasma was sufficient, in the solutions used, to delay the action of two drops of thrombin on the fibrinogen usually for an hour or more or to prevent clotting altogether In the specimens containing 3 4 and 5 drops of thrombin the delay was proportionally less marked the specimen containing 5 drops of thrombin clotting usually in five minutes It is easy by this method to determine the relative amounts of antithrombin in different specimens of blood In bird's plasma or the plasma from a peptonized dog the antithrombin exists in relatively large amounts so that a drop of the heated plasma may inhibit completely the action of such amounts of thrombin as are here considered

In determining the time of coagulation in the different specimens the tubes containing the mixtures were examined, usually at intervals of five minutes, by gently inclining the tubes When the thrombin was in distinct excess the contents of the tubes set to a firm clot, but when the thrombin was nearly neutralized by the antithrombin it was difficult or often impossible to ascertain when clotting occurred, since under such circumstances the clot forms in several stages First, a delicate veil which on agitation shrinks to a small membrane while later a more solid gelatinous clot may form

2 The solutions of thrombin should not be filtered since the thrombin is absorbed to some extent by the filter paper

Specimens in which the thrombin and antithrombin existed in this balanced proportion were useless for comparative purposes, and in such cases it was necessary to rely on the specimens containing more thrombin in which the clotting occurred promptly

By way of illustration of the method, the results of one of the comparisons made between hemophilic and normal blood may be quoted

Heated Plasma Drops	Thrombin Drops	Time Interval Minutes	Fibrinogen Drops	Coagulation Minutes
		NORMAL INDIVIDUAL		
1	2	15	10	160
1	3	15	10	10
1	4	15	10	10
1	5	15	10	5
		HEMOPHILIC BOY		
1	2	15	10	*
1	3	15	10	35 40
1	4	15	10	15
1	5	15	10	10

* No clot in seven hours

This method has been applied to the examination of a very large number of specimens of human blood, normal and pathological It has been found that while the content in antithrombin of normal blood exhibits certain fluctuations the variations are not large and do not necessarily run parallel to the coagulation time, owing to the fact that this latter time depends upon the balance between the thrombin and the antithrombin In pathological bloods no significant constant variation in the amount of antithrombin has been detected except possibly in the condition known as idiopathic thrombosis

Prothrombin.—In order to arrive at an idea of the relative amount or relative efficacy of the prothrombin in the plasmas examined I have adopted the procedure of adding to the oxalated plasma the optimum amount of calcium chlorid, that is to say, the amount that causes the most rapid clotting This method was suggested by the observation that in a successful peptonized plasma (dog) addition of calcium chlorid does not cause clotting even when the plasma has been diluted previously with water If, however, such a plasma is first oxalated and then calcium chlorid is added in proper amounts, clotting occurs readily For some reason, not easily understood at present, the act of oxalating, that is, the decalcifying of the solution, intensifies the activation caused by the subsequent addition of calcium, so that the end result is apparently a greater production of thrombin, sufficient in fact to overcome the antithrombin and cause clotting The alternative hypothesis that suggests itself, namely, that the process of oxalating may weaken the antithrombin, is easily shown to be erroneous In some way the decalcification and subsequent calcification serve to bring into active form the maximum supply of prothrombin present in the blood Whether or not this interpretation of the reaction is correct the simple procedure described has brought out a very constant and striking difference between normal and hemophilic blood The reaction was carried out as follows Five drops of oxalated plasma were placed in each of four tubes and to these were added in series, 2, 3, 4 and 5 drops of a 0 5 per cent solution of calcium chlorid, with the idea that in one of the tubes the optimum concentration of calcium would be obtained With the amount of plasma used clotting occurred first in the tubes containing 2 or 3 drops of calcium chlorid With one drop the clotting occurred slowly or not at all, owing to an insufficiency of calcium, while in the tubes with 4 or 5 drops the clotting was somewhat slower than in the tubes with 2 or 3 drops owing to too great an excess of calcium Tested by this method the clotting of normal blood plasma is remarkably uniform, varying between nine and twelve minutes In the plasmas

of hemophilic blood, on the contrary, the time of clotting is greatly prolonged This fact, indeed, has been noted by Addis,[3] although he does not seem to have used the reaction in any extensive way I have employed the reaction, as described above, for a large series of bloods to throw light on the amount or efficacy of the prothrombin, when parallel tests have established the fact that there is no variation in the amount of antithrombin in the bloods examined

RESULTS

Purpura—At different times in the course of this work specimens of blood have been obtained from patients suffering from various forms of purpura, including one case of severe purpura hemorrhagica The time of coagulation of these bloods, when obtained by venepuncture, did not vary distinctly from normal, and the content of the plasmas in antithrombin and prothrombin was also within the limits found for normal bloods It does not seem desirable at present to publish the details of these negative findings, since this paper deals especially with hemophilia and thrombosis Duke[4] has published some interesting observations on cases of purpura hemorrhagica which show that the coagulation time may be normal while the bleeding time is greatly prolonged By the latter term he designates the time that a small wound will continue to bleed, as determined by pressing against it a bit of filter paper at definite intervals of time He has connected the increased bleeding time in purpura with a marked deficiency in platelets in consequence of which platelet thrombi fail to form in the wounded vessels My work throws no light on this point, but it does demonstrate that severe purpura hemorrhagica may exist without any detectable abnormality in the factors concerned in the clotting of the blood The tendency to bleeding in these cases cannot be referred apparently to a deficient power of coagulation

Hemophilia—I have had the opportunity of examining with care two cases of undoubted hereditary hemophilia and a third case of a young boy in which no history of inheritance could be obtained The history of these cases is as follows:

REPORT OF CASES

CASES 1 and 2—Joseph Y, aged 12 years, and Jesse Y, aged 8 years, sons of Joseph Y of Baltimore, a Russian Jew The hemophilic tendency in these children was inherited from the mother She stated that three of her brothers died from hemorrhage, one at 17 years of age as the result of an operation on the tonsils, one at 6 years from a cut on the face made accidentally by a playmate, and one at the time of circumcision She states also that a cousin, daughter of her father's brother, has married and has hemophilic boys An effort to locate this latter family was not successful In the present family of Joseph Y there

3 Addis Jour Pathol and Bacteriol, 1911, xv, 436

4 Duke Jour Am Med Assn, 1910, lv, 1185, Bull Johns Hopkins Hosp, 1912, xxiii, 145

are seven children, three boys, Joseph, 12 years, Jesse, 8 years and Fred, 5 years, all markedly hemophilic, and four girls none of whom have exhibited any tendency to bleed Great difficulty from bleeding has been experienced at various times with the three boys All have suffered from serious hematomata due to accidents in playing Joseph at the time of circumcision is said to have bled for a week At 4 years of age he was brought to the Johns Hopkins Hospital with hemorrhage caused by biting the tongue while eating an apple The hemorrhage at first profuse, lasted nearly a week It was partially controlled by cauterizing with lunar caustic Parents gave a history of bleeding spells in the boy from slight injuries on four or five occasions The coagulation time as then recorded was only six to twelve minutes The method is not stated but probably the blood was taken by puncture through the skin and the result is therefore not reliable He has been a patient at the hospital since this time on several occasions in connection with joint troubles Fred when 3½ years of age was taken to the Hebrew Hospital, Baltimore, on account of a persistent hemorrhage from the tonsils supposed to be due to an accident Bleeding continued for several days and was controlled finally or ceased finally after an injection of horse serum (8 c c)

In the experiments here reported only Joseph and Jesse were used as they were old enough to come to the laboratory and could be used conveniently for the operation of venepuncture

CASE 3.—A H S, 4 years old, patient in the Harriet Lane Home of the Johns Hopkins Hospital Since his third month difficulty has been experienced with bleeding from small cuts and injuries of any kind have been followed by "black and blue" areas in the region involved At the time of admission to the hospital—March 25, 1913—the child was very anemic red blood cells, 1,730,000, white blood cells, 15,300, Hb 33 per cent, platelets apparently normal, although no count was made Large purpuric areas existed on right arm and leg, right shoulder, left tibia and right cheek Right arm, hand and shoulder much swollen, right leg also swollen The child remained in the hospital until May 1, 1913, and showed very marked improvement A blood count made April 15, showed red blood cells 4,270,000, white blood cells 13,000 and Hb 80 per cent On three occasions human blood serum was injected subcutaneously, on April 12, 10 c c, April 14, 14 c c and April 24, 15 c c Through the kindness of Dr John Howland I was given an opportunity to examine the blood on two occasions, once, the day after admission to the hospital and once just before his discharge On each occasion the specimens of blood were obtained for me by Dr W L Moss to whose kindness and skill I have been much indebted throughout this work

COAGULATION TIME OF THE HEMOPHILIC BLOOD

The coagulation time was determined on specimens of 2 or 4 c c of blood obtained directly from the vein by means of the sterilized syringe and expelled at once into wide tubes (diameter 21 mm), which had been cleaned carefully with a bichromate acid mixture The clotting time was estimated up to the time that the clot was firm enough to allow the tube to be inverted Comparisons were always made with specimens of blood taken from normal individuals by the same method Tested by this method the time of clotting of normal blood varies usually between twenty and forty minutes The time shown by the hemophilic blood at different periods was as follows

Nov 2, 1912	Joseph Y	Clot apparent in 3 hours, complete in 4 hours
	Jesse Y	Clot apparent in 3 hours, complete in 4 hours

Dec 21, 1912	Joseph Y	Clot apparent in 4 hours, complete in 5 hours
	Jesse Y	Clot apparent in 4 hours, complete in 5 hours
Jan 18, 1913	Jesse Y	Clot apparent in 3 hours, complete in 4 hours
April 19, 1913	Joseph Y	Clot apparent in 4 hours, complete in 5 hours
	Jesse Y	Clot apparent in 4 hours complete in 5 hours
April 28, 1913	Joseph Y	Clotted between 2 and 2½ hours
May 17, 1913	Joseph Y	Clotted in 3½ hours
June 14 1913	Joseph Y	Clotted in 4 hours
March 26, 1913	A H S	Clotted between 2½ and 3 hours
May 1 1913	A H S	Clotted between 3 and 4 hours

It is apparent from these data that in the three cases examined there was a marked delay in the spontaneous coagulation of the hemophilic blood such as has been reported by several other observers So far as could be determined from the examinations made of the blood of the brothers Y extending over a period of eight months there was at no time an approach to normal and in fact but little indication of any variation The deficient coagulability was due to some permanent alteration in the properties of the blood It is noteworthy in the case of A H S that in spite of the fact that he had received during his stay in the hospital three injections of human serum and that his general condition had improved so greatly, the coagulation time at the end was as long or longer than when he first came into the hospital In regard to the coagulation time of blood, it should be emphasized that error may arise easily if the technic of obtaining the specimens fails in any respect Constant and reliable results are obtained only in those cases in which the needle of the syringe enters the vein readily on the first puncture When the vein is missed or transfixed and the lumen is found only after several trials, there is a likelihood that the specimen of blood may contain some tissue-juice, and therefore clot more rapidly than it would alone This effect was shown very distinctly on one occasion with Jesse Y On the first attempt the needle missed the vein, and only after several trials was it possible to draw about 2 c c of blood When emptied into a tube this specimen clotted in ten minutes A specimen of 8 c c was then obtained from the vein of the other arm The technic was good, and a specimen of 2 c c placed in a tube clotted in the usual time, namely, between four and five hours

No difficulty from hemorrhage was experienced in taking blood from the hemophilic patient, provided the vein was not wounded On two

5 In this case 1 c c only was taken for examination, and there is a probability that some tissue juice was mixed with this specimen, since difficulty was experienced in getting into the vein

occasions when the needle did not enter promptly, and there was probably some laceration of the vein there developed later a somewhat extensive purpuric area which persisted for a long period

EFFECT OF THROMBOPLASTIC SUBSTANCE AND OF CALCIUM ON THE CLOTTING OF HEMOPHILIC BLOOD

As stated above, admixture with thromboplastic material of the tissues accelerates greatly the time of clotting On several occasions this result was obtained with the active principle of the tissue extracts that is to say, with the phosphatid (kephalin) extracted from brain tissue as described in a previous paper [6] In one experiment the kephalin was dissolved in a 6 per cent solution of dextrose and a control was made with a similar solution of dextrose without kephalin The kephalin caused a marked acceleration in the clotting of both normal and hemophilic blood

Normal blood 1 c c blood plus 1 c c dextrose solution (6 per cent) clotted in 10 minutes
1 c c blood plus 1 c c of dextrose solution containing kephalin clotted in five minutes

Hemophilic blood (Joseph Y) 1 c c blood plus 1 c c dextrose solution clotted between two hours, ten minutes, and three hours, thirty minutes
1 c c blood plus 1 c c dextrose solution containing kephalin clotted in ten to eleven minutes

A similar result was obtained with specimens taken from Jesse Y

In a second experiment the coagulation time of a mixture of blood with kephalin was compared with that of the undiluted blood The specimens containing the kephalin were placed in narrow centrifugal tubes which would tend also to shorten the time

Coagulation time of 2 c c blood Normal 30 minutes, Joseph Y, four to five hours, Jesse Y, four to five hours

Coagulation time of 2 c c blood plus 1 c c dextrose solution containing kephalin Normal, four to five minutes, Joseph Y, twelve minutes, Jesse Y, eleven minutes

Similar results were obtained in a third experiment, in which the kephalin was added in aqueous solution, and in which consequently there was considerable hemolysis of the blood

It is stated that the content in calcium of the hemophilic blood is normal (Nolf[7]), and that addition of calcium chlorid does not hasten the time of coagulation (Morawitz and Lossen[8]) In my experiments it was noted that the oxalated hemophilic blood gave a good deposit of

6 Howell Am Jour Physiol, 1912, xxxi, 1

7 Nolf Eine neue Theorie der Blutgerinnung Ergebn d inn Med, 1913, x, 275

8 Morawitz and Lossen Deutsch Arch f klin Med 1908, xciv 110

calcium oxalate on centrifugalization, but the deposit to the eye seemed less than that obtained from similar specimens of normal blood An attempt was made to determine the amount of calcium in small specimens of the blood (8 c c) but the results were unsatisfactory In a single experiment it was found that addition of calcium chlorid to the hemophilic plasma hastened somewhat the time of coagulation, as follows

2 c c hemophilic plasma (Joseph Y), coagulation time = four to five hours

1 c c hemophilic plasma + 3 drops CaCl ½ per cent, coagulation in two hours thirty minutes

1 c c hemophilic plasma + 6 drops CaCl ½ per cent coagulation in two hours, ten minutes

ANTITHROMBIN IN HEMOPHILIC BLOOD

Examinations were made of the amount of antithrombin by the method previously described In the case of the brothers Y, these examinations were made at irregular intervals during a period of several (eight) months, the blood being compared each time with a specimen of normal blood In all cases the examination was made as soon as possible after taking the specimen from the vein, since experience had shown that when the blood is kept there is a gradual deterioration in the amount of antithrombin The results of these comparisons are given in the accompanying table It will be understood that the procedure was as previously described that is to say, the clear plasma of the blood was heated to 60 C and filtered One drop of this heated plasma was then added to 2 3, 4 and 5 drops of a thrombin solution and, after an interval of fifteen minutes ten drops of the fibrinogen solution (dried plasma) were placed in each tube Owing to variations in the strengths of the thrombin solutions and fibrinogen solutions used, the different experiments are not comparable among themselves In each experiment the antithrombin in the hemophilic plasma is to be compared with the normal blood taken at the same time and submitted to the same procedure

Examination of these results shows that in general the amount of antithrombin in the hemophilic blood was somewhat greater than in the normal specimen with which it was compared The excess, however, was not large and in some cases was not distinctly shown (experiments of December 21, April 19, April 28, May 17) Since in these latter cases the long coagulation time was still exhibited by the blood, it seems most probable that the characteristic delay in coagulation shown by hemophilic blood cannot be referred to an increase in the absolute amount of antithrombin present in the blood As will be shown, however, in the next paragraph, there is good reason to believe that in these bloods there is always a relative excess of antithrombin, which is quite sufficient to account for the prolonged period of coagulation

TABLE 1.—Results of Examination for Antithrombin in Hemophilic Blood

Date	Name	Thrombin in Drops	Time of Coagulation
Oct 19, 1912	Joseph Y (hemophilic)	2	8 to 15 hours
		3	45 to 50 minutes
		4	20 minutes
		5	15 minutes
	John S (normal)	2	2 to 8 hours
		3	30 to 35 minutes
		4	10 minutes
		5	10 minutes
Nov 2, 1912	Joseph Y (hemophilic)	2	4 to 18 hours
		3	45 minutes
		4	35 minutes
		5	15 minutes
	Jesse Y (hemophilic)	2	45 minutes
		3	20 minutes
		4	15 minutes
		5	10 minutes
	W H H (normal)	2	20 minutes
		3	10 minutes
		4	10 minutes
		5	5 minutes
Nov 16, 1912	Joseph Y (hemophilic)	2	6 to 15 hours
		3	3 to 6 hours
		4	30 minutes
		5	20 minutes
	Jesse Y (hemophilic)	2	6 to 15 hours
		3	3 to 6 hours
		4	30 minutes
		5	20 minutes
	D R H (normal)	2	3 to 6 hours
		3	30 minutes
		4	15 to 20 minutes
		5	10 minutes
Dec 21, 1912	Joseph Y (hemophilic)	2	No clot in 24 hours
		3	Begun in 1 hour
		4	20 minutes
		5	10 minutes
	Jesse Y (hemophilic)	2	No clot in 24 hours
		3	Begun in 2 hours
		4	20 minutes
		5	15 minutes
	H, hospital patient (normal) ?	2	45 minutes
		3	15 minutes
		4	10 minutes
		5	10 minutes
	D (normal)	2	No clot in 24 hours
		3	?
		4	40 minutes
		5	20 minutes
Jan 18, 1913	Joseph Y (hemophilic)	2	No clot in 3 hours
		3	15 minutes
		4	10 minutes
		5	5 minutes
	Jesse Y (hemophilic)	2	No clot in 3 hours
		3	20 minutes
		4	10 minutes
		5	5 minutes
	M (normal)	2	1½ to 2 hours
		3	10 minutes
		4	5 minutes
		5	5 minutes

TABLE 1.—RESULTS OF EXAMINATION FOR ANTITHROMBIN IN HEMOPHILIC BLOOD.—(Continued)

Date	Name	Thrombin in Drops	Time of Coagulation
April 19, 1913	Joseph Y (hemophilic)	2	20 to 25 minutes
		3	10 minutes
		4	5 minutes
		5	5 minutes
	Jesse Y (hemophilic)	2	20 to 25 minutes
		3	10 minutes
		4	5 minutes
		5	5 minutes
	John S (normal)	2	15 to 20 minutes
		3	10 minutes
		4	5 minutes
		5	5 minutes
April 28, 1913	Joseph Y (hemophilic)	2	10 minutes
		3	5 minutes
		4	5 minutes
		5	5 minutes
	John S (normal)	2	10 minutes
		3	5 minutes
		4	5 minutes
		5	5 minutes
May 17, 1913	Joseph Y (hemophilic)	2	10 minutes
		3	10 minutes
		4	5 minutes
		5	3 minutes
	John S (normal)	2	10 minutes
		3	5 minutes
		4	3 minutes
		5	3 minutes
March 26, 1913	A H S (hemophilic)	2	Small membrane at 25 minutes
		3	10 minutes
		4	5 minutes
		5	5 minutes
	John S (normal)	2	Small membrane at 20 minutes
		3	5 minutes
		4	5 minutes
			5 minutes
May 1, 1913	A H S (hemophilic)	2	?
		3	15 minutes
		4	10 minutes
		5	5 minutes
	George S (normal)	2	?
		3	5 minutes
		4	5 minutes
		5	5 minutes

PROTHROMBIN IN HEMOPHILIC BLOOD

The relative amounts of prothrombin in the hemophilic and normal bloods were determined by the simple method, described above, of first oxalating the blood and then adding the optimum amount of calcium chlorid to the clear plasma. This procedure was carried out on a great many specimens of blood, normal and pathological. It may be said in

general that the time of coagulation for normal human blood is quite constant, averaging about ten minutes, with variations from eight or nine to twelve or thirteen minutes In normal dog's blood the time of coagulation is somewhat shorter, averaging about six minutes, with variations between five and nine or ten minutes In the hemophilic bloods the results were entirely different, as shown by Table 2

TABLE 2.—RESULTS OF EXAMINATION FOR PROTHROMBIN IN HEMOPHILIC BLOOD

Date	Name	Calcium Chlorid, 0 5 Per Cent, in Drops	Time of Coagulation (5 Drops of Oxalated Plasma)
March 26, 1913	A H S (hemophilic)	1	No clot
		2	90 minutes
		3	120 to 150 minutes
		4	120 to 150 minutes
	John S (normal)	1	No clot
		2	9 minutes
		3	12 minutes
		4	14 minutes
May 1, 1913	A H S (hemophilic)	2	?
		3	Partly clotted in 3 hrs
		4	Partly clotted in 3 hrs
		5	?
	George S (normal)	2	10 minutes
		3	14 minutes
		4	20 minutes
		5	30 minutes
April 19, 1913	Joseph Y (hemophilic)	2	No clot in 4 hours
		3	No clot in 4 hours
		4	No clot in 4 hours
	Jesse Y (hemophilic)	2	Clot in 2 to 2½ hours
		3	Clot in 2 to 2½ hours
		4	Clot in 2½ hours
	John S (normal)	2	10 minutes
		3	12 minutes
		4	18 minutes
April 28, 1913	Joseph Y (hemophilic)	2	Clot later than 5 hours
		3	Clot in 5 hours
		4	Clot later than 5 hours
	John S (normal)	2	11 minutes
		3	13 minutes
		4	17 minutes
May 17, 1913	Joseph Y (hemophilic)	2	Clot in 3 hours to 3 hours, 10 minutes
		3	Clot in 3 hours to 3 hours, 10 minutes
		4	Clot later than 3½ hrs
	John S (normal)	2	8 minutes
		3	10 minutes
		4	12 minutes

As will be seen from examination of the table, the optimum amount of calcium in the normal was obtained with two drops of the solution

of calcium chlorid With this amount of calcium the clotting time of the normal plasma was, in the different experiments, 9, 10, 10, 11 and 8 minutes The clotting time for the hemophilic bloods was 90, 180+, 240+, 120, 300, 180+ minutes

The much delayed coagulation of hemophilic plasma under these conditions may be accounted for theoretically in several ways It may be due, in the first place, to the presence of an excessive amount of antithrombin The observations reported above give but little support to this explanation The amount of antithrombin in hemophilic plasma may be somewhat greater than that found in normal blood, and according to my results, is never distinctly less than normal, but on the other hand, when it is present in normal amounts, the hemophilic blood still exhibits in a marked way the characteristic delay in coagulation when oxalated and again recalcified In other words, there is no parallelism between the amount of antithrombin and the delay in coagulation, and we cannot therefore explain the latter phenomenon by reference to variations in the antithrombin In the second place, the delayed coagulation of the hemophilic blood may be referred, as has been done by Sahli[9] and by Morawitz and Lossen, to a deficiency in thromboplastic substance, or rather to that particular form of thromboplastic substance which Morawitz has designated as thrombokinase There is no satisfactory method of detecting variations in quantity of the thrombokinase The authors named have arrived at the conclusion that thrombokinase is lacking in hemophilic blood (and hemophilic tissues), mainly by a process of exclusion of other possible explanations I believe, and have given experimental reasons for the belief, that such a thing as thrombokinase does not exist Morawitz's ingenious and attractive hypothesis in regard to this substance has proved to be an obstacle rather than an assistance in the study of the phenomenon of clotting The reasons given for the existence of a kinase in the intestine with the property of activating trypsin, are convincing, since much in the way of direct proof is easily obtained Any one may demonstrate that the mucous membrane of the intestine contains an organic substance which effects the rapid activation of trypsin But no such proof is obtainable for the existence of a thrombokinase From an experimental standpoint the whole matter is complicated here by the additional hypothesis that the kinase alone cannot activate thrombin, but requires the coactivity of calcium That calcium is concerned in the activation of prothrombin to thrombin is beyond question, but every proof furnished to demonstrate that calcium alone is concerned in this activation is met by the objection that the kinase also is present in the solutions Since this kinase has no known properties or reactions except that of assisting calcium in the activation

[9] Sahli Deutsch Archiv f klin Med, 1910, xcix, 518

of thrombin, the argument assumes the form of a perfect circle Kinase is necessary to the formation of thrombin, hence if thrombin is formed a kinase is present What is usually designated as a thrombokinase in the blood and other tissues is in reality the substance or substances which facilitate the process of clotting, and which have long been known under the terms zymoplastic or thromboplastic substances According to my work, they consist essentially of a special phosphatid (kephalin?), which accelerates the process of clotting, not by any direct action on prothrombin, after the manner of a kinase, but by its neutralizing effect on the antithrombin In the general sense of the term it is a thromboplastic substance, but not a thrombokinase Whether or not this material is lacking in hemophilic plasma, I have not been able to determine in any wholly satisfactory way Addis[3] reports certain experiments which he believes demonstrate that as regards this factor there is no difference between normal and hemophilic plasma, but his experiments do not seem to me to be conclusive Sahli, on the other hand, reports experiments on the thromboplastic effect of washed blood corpuscles from normal and from hemophilic blood, which seem to show that the former contain more thromboplastic material than the latter But this conclusion, if correct, is scarcely applicable to the case under discussion The thromboplastic substance liberated in the blood after shedding comes, it is assumed usually, from the disintegration of the platelets When blood is received at once into oxalate solutions, this disintegration is prevented The platelets are fixed, indeed, permanently fixed When the blood is centrifugalized they are thrown down unchanged, as may be shown by microscopic examination, and subsequent treatment with water or saline solutions shows that they now possess a rigidity altogether different from their original condition in the normal plasma It is probable, therefore, that when the normal or hemophilic blood is oxalated immediately and centrifugalized, the clear plasma obtained has not received an accession of thromboplastic substance from disintegrating platelets Nevertheless under these conditions, addition of calcium to such plasmas is followed by prompt and uniform clotting in the normal plasma and by a much delayed and variable clotting time in the hemophilic plasma My own explanation of the cause of this difference is similar to that proposed by Addis, namely, that the trouble lies in a variation in the prothrombin This view is discussed further in the succeeding paragraph Outside of all theoretical considerations, it is well to emphasize the fact that the simple procedure described above, of oxalating plasma and then recalcifying by adding calcium chlorid, gives a convenient method of detecting hemophilic blood and of distinguishing it from the bloods of other hemorrhagic conditions

THE CAUSE OF HEMOPHILIA

In the recent admirable monograph on hemophilia by Bulloch and Fildes,[10] the condition is defined as "an inherited tendency in males to bleed" The definition is unsatisfactory, in that it fails to suggest a cause for the condition, and possibly also in that it lays too much stress on the hereditary feature There is no question, of course, that hemophilia is transmissible by heredity, and that most of the undoubted cases reported exhibit this history, but on the other hand it cannot be denied at present that cases may arise spontaneously To exclude such cases from the group of hemophilia because there is no evidence of a similar condition in the family records, is not justifiable In all probability such spontaneous cases may in turn be transmitted according to the law controlling this inheritance Outside its transmissibility as a sex-limited inheritance, hemophilia is characterized clearly by certain reactions or properties of the blood which distinguish it from other hemorrhagic conditions in which there is also a "tendency to bleed" The main distinguishing feature of the hemophilic condition is the greatly delayed coagulation time of the blood, when the blood is removed from the vessels without coming in contact with the tissues This peculiarity is demonstrated more easily and certainly, in contrast with other bloods, if the specimen of blood is first oxalated, then centrifugalized to obtain a clear plasma and then recalcified by the addition of a suitable amount of calcium chlorid If the specimen so treated should show a coagulation time exceeding that of the normal blood (nine to twelve minutes), there would be reason for claiming the presence of a hemophilic condition, and the gravity of the condition would be in proportion to the length of time required for coagulation

All recent workers agree on this delayed or deficient coagulability as the characteristic feature of hemophilia and attempts to explain the proximate or ultimate cause of the condition start from this point The nature of the explanations offered varies of course with the theory of coagulation adopted Morawitz and Lossen and Sahli, adopting the theory of coagulation proposed by Morawitz, have been led to believe that the defect of the hemophilic blood consists in a deficiency or lack of thrombokinase, Weil[11] has suggested that the delayed coagulation may be caused by the presence of some inhibiting body (antithrombin), Wright[12] has attributed the trouble, in part at least, to a deficiency in calcium, Nolf, in accordance with his special theory of coagulation, supposes that there is a quantitative or qualitative defect in the factor of coagulation designated by him as thrombozym Addis in his series of

10 Bulloch and Fildes Hemophilia, Eugenic Laboratory Memoirs, University of London, 1911, xii

11 Weil Presse méd, 1905, p 672, Bull et mem Soc méd d hôp de Paris, Oct 26, 1906

12 Wright Brit Med Jour, 1893, p 223

thoughtful experiments has come to the conclusion that the essential difficulty lies in a change in the properties of the prothrombin My own work has convinced me that the antithrombin theory of hemophilia is incorrect, in spite of the fact that my experiments were begun in the expectation of supporting this view There is little or no positive evidence in favor of the view that a deficiency of calcium is the fundamental trouble, and I am persuaded that the explanations offered by Morawitz, Sahli and Nolf have been influenced by an incorrect theory of the process of clotting Sahli's generalization that in the hemophilic there is a loss of thromboplastic power (thrombokinase), not only in the corpuscles of the blood, but in the tissues at large, is certainly not applicable in all cases The slow clotting hemophilic blood may coagulate quite promptly if it is brought into contact with the tissue juices of the external tissues There are at present, so it seems to me, no positive facts supporting this general view of a defect in thromboplastic material in the hemophilic blood My own experiments have led me to believe that the trouble lies, where it was traced by Addis, in the factor of prothrombin, that is to say, the material from which the thrombin is formed The experiments described above on the coagulation time of oxalated blood after the addition of calcium can only mean that this prothrombin material is deficient in quantity or altered in character Addis has adopted the latter view He believes that the prothrombin of the hemophilic blood is present in normal amounts, but is so altered in properties as to require a longer time than normal for activation to thrombin This explanation has seemed to me to be improbable, because, in the first place, I have found, as reported in this paper, that addition of thromboplastic (phosphatid) material accelerates greatly the time of clotting of the hemophilic blood Addis admits this fact, but claims that it is true only when large amounts of this material are used Assuming that this latter qualification is correct, it does not affect the main conclusion that the prothrombin possesses its normal properties in relation to activation by calcium, since the thromboplastic material is effective only by virtue of the fact that it removes the antagonistic influence of the antithrombin However, the point that Addis raises can be settled only by isolating the prothrombin from the hemophilic plasma and testing the action of calcium on it in comparison with prothrombin obtained by the same method from normal blood

I have been able, fortunately, to obtain the prothrombin from blood plasma by the simple method which I have employed already for the isolation of thrombin The method is as follows Blood is obtained from a vein of the arm by means of a syringe and is oxalated at once (8 c c. of blood to 1 c c of a 1 per cent solution of sodium oxalate made up in sodium chlorid solution 0 9 per cent) The specimen is centrifugalized and the clear plasma is drawn off To this plasma is added an equal

volume of acetone and the precipitate is filtered off through a small filter The filter paper is opened, the precipitate is spread to a thin layer and is then dried rapidly in a current of air from an electric fan The dried filter paper with its adherent precipitate is cut into small pieces and is covered with water (8 or 10 c c) and allowed to stand for about half an hour The extract is then filtered The opalescent solution contains prothrombin, as can be shown most satisfactorily by its reaction with fibrinogen solutions The fibrinogen solutions used for this purpose I have prepared from the plasma of cat's blood by a modification[13] of Hammarsten's method of repeated precipitation by half saturation with sodium chlorid If the fibrinogen is precipitated three times the final solution in 0 9 per cent sodium chlorid (with a trace of sodium bicarbonate if necessary) gives no coagulation at all on the addition of calcium, but clots readily with thrombin With such solutions of fibrinogen, or indeed with the less pure solutions obtained by one or two precipitations, the presence of prothrombin in the solutions described above may be demonstrated very readily by experiments of which the following is an example

1 Fibrinogen solution, 10 drops + $CaCl_2$, ½ per cent, 1 drop = no clot in twenty-four hours

2 Fibrinogen solution, 10 drops + prothrombin solution 3 drops = no clot in twenty-four hours

3 Fibrinogen solution 10 drops + prothrombin solution 3 drops + $CaCl_2$, ½ per cent, 1 drop = clot in 2 to 3 minutes

It should be stated that fibrinogen solutions prepared as described are not constant in their reactions In some cases they clot very readily with thrombin, in other cases the clotting requires a longer time, and on standing for twenty-four hours or more the fibrinogen may undergo a modification of some kind so that it loses the property of yielding a clot on the addition of thrombin In all cases, however, the freshly prepared fibrinogen may be used to demonstrate the presence of the prothrombin, since the solutions of the latter have no effect on the fibrinogen until calcium is added, and then the thrombic action is clearly shown In order to determine whether or not the prothrombin from the hemophilic blood differs in its reaction to calcium from that of normal blood, I have prepared the prothrombin from one of the hemophilic subjects used in this investigation (Joseph Y) and compared it with the prothrombin from a normal blood The following results were obtained

Experiment, Oct 19, 1913 —Eight cubic centimeters of blood taken from Joseph Y and 8 c c from a normal (John S) Each specimen was oxalated and treated with acetone as described above The aqueous solutions of prothrombin were tested on a freshly prepared fibrinogen solution

Control Experiments —Fibrinogen solution, 10 drops + water 5 drops + $CaCl_2$ (½ per cent) 1 drop = no clot in twenty-four hours

Fibrinogen solution, 10 drops + prothrombin solution 5 drops = no clot in twenty-four hours

13 Howell Am Jour Physiol, 1910, xxvi, 461

Prothrombin from Normal Blood—Fibrinogen solution 10 drops + prothrombin solution 5 drops + $CaCl_2$, 1 drop = clot in twenty eight minutes

Fibrinogen solution 10 drops + prothrombin 3 drops + $CaCl_2$, 1 drop = clot in 50 to 60 minutes

Prothrombin from Hemophilic Blood—Fibrinogen solution 10 drops + prothrombin solution 5 drops + $CaCl_2$, 1 drop = clot in twenty minutes

Fibrinogen solution 10 drops + prothrombin solution 3 drops + $CaCl_2$, 1 drop = clot in fifty minutes

This experiment, which was repeated several times with similar results, demonstrates quite definitely that the separated prothrombin from hemophilic blood yields active thrombin in the presence of calcium quite as readily as the prothrombin from normal blood The qualitative difference claimed by Addis does not exist In explaining the delayed coagulation of the hemophilic blood we must assume, it seems to me, that the prothrombin is present in subnormal amount in the circulating blood Since the antithrombin, on the contrary, exists in normal or even in sligthly supranormal amounts, the delay in coagulation of the shed blood is readily explained On the basis of the facts presented in this paper, I would conclude that the essential condition in hemophilic blood which is immediately responsible for its delayed coagulation is a subnormal amount of prothrombin in the circulating blood

Hemophilia may be defined as a condition, limited to the male, in which the coagulation time of the blood is markedly prolonged in consequence of a deficiency in the amount of the contained prothrombin, with the additional characteristic that the defect is transmissible by heredity in accordance with the so-called law of Nasse

The ultimate cause of this condition cannot be stated So far as we know, the prothrombin present in blood-plasma is furnished by the blood-platelets, and it is reasonable to assume that the defect in question is referable to some functional change in these elements

THROMBOSIS

During the course of this work the opportunity has offered to examine several cases of thrombosis in which there were no obvious mechanical or inflammatory conditions to account for the trouble The cases are few in number, and unfortunately the examination was not complete in each case, but the results seem to be suggestive

CASE 4.—Dr H, just recovering from mild attack of thrombosis involving the saphenous and the abdominal veins Some soreness and edema over region involved He had had several similar attacks at intervals of some weeks Specimen of 8 c c of blood taken from an arm vein and compared with similar specimen from a laboratory attendant (John S)

The results of this test indicate that the blood of the thrombotic patient contained less antithrombin than the normal with which it was compared, and, indeed less than any of the normals used in any of these experiments

Coagulation time (4 c c) Patient, 29 to 30 minutes
Normal, 38 to 39 minutes

ANTITHROMBIN TEST

Heated Plasma Drops	Thrombin Drops	Time Interval Minutes	Fibr Sol Drops	Coagulation Minutes
		Normal		
1	2	15	10	40
1	3	15	10	10
1	4	15	10	5
1	5	15	10	5
		Patient		
1	2	15	10	10
1	3	15	10	5
1	4	15	10	5
1	5	15	10	5

CASE 5.—Charles G., patient in the Johns Hopkins Hospital, service of Dr Barker. Aged 52. Onset of attack one month previous to admission, dyspnea on exertion, pain in left side, swelling of feet. Physical findings: dyspnea, hydrothorax, mitral and aortic insufficiency. Blood-pressure, 140. Blood count, r. b. c., 4,300,000, w. b. c., 7,400, Hb., 90 per cent. The day before the blood was examined the patient developed a marked swelling of the whole left leg, diagnosed as due to thrombus in the left femoral or iliac vein. Some of the superficial veins at left elbow firmly thrombosed.

Specimen of blood, 10 c.c., taken from arm vein and compared with a similar specimen from one of the laboratory attendants (M.).

Coagulation time (2 c.c.) Patient, 8 minutes
Normal, 41 minutes

ANTITHROMBIN TEST

Heated Plasma Drops	Thrombin Drops	Time Interval Minutes	Fibr Sol Drops	Coagulation Minutes
		Normal		
1	2	15	10	*
1	3	15	10	10†
1	4	15	10	5
1	5	15	10	5
		Patient		
1	2	15	10	5
1	2	15	10	2
1	2	15	10	2
1	2	15	10	2

* First membrane at twenty minutes.
† Partial.

In this case also the amount of antithrombin was distinctly less than in the normal. In fact the clotting with the thrombotic plasma was so rapid as to indicate the practical absence of antithrombin in its heated plasma.

PROTHROMBIN TEST

Oxalated Plasma Drops	$CaCl_2$, ½ Per Cent Drops	Coagulation Minutes
	Patient	
5	2	7
5	3	7
5	4	9
5	5	11
	Normal	
5	2	10
5	3	14
5	4	17
5	5	19

The more rapid clotting of the oxalated plasma of the thrombotic blood may have been due to a greater amount of prothrombin or to a

diminished amount of antithrombin The previous test for antithrombin indicates that the latter explanation is probably the correct one, and this conclusion is borne out by a subsequent experiment in which the serum from the patient and from the normal were compared in regard to their strength in thrombin, the precaution being taken of adding to each an equal volume of kephalin-solution to neutralize the antithrombin present This test gave the following result

COMPARISON OF STRENGTH IN THROMBIN

Fibrinogen Solution Drops	Serum and Kephalin Drops	Coagulation Minutes
	Patient	
10	2	12
10	3	6
10	4	5
10	5	3
	Normal	
10	2	10
10	3	5
10	4	5
10	5	3

According to this test there was practically no difference in the amount of thrombin in the two specimens of serum

CASE 6.—Mrs N, private patient of Dr Thayer History of two attacks of thrombosis — one six years previously with evidence of thrombosed veins in calf, groin and inner side of leg, one beginning four months before examination and following attack of measles There were pain and tenderness over left femoral vein, knots in groin and under skin of legs, first on left side, later on right side In bed for three months On one occasion when convalescing there was a sudden attack of severe pain in side, with marked dyspnea A similar attack followed after an interval of some time Examination of the blood was made four months after beginning of last attack when the patient had recovered and showed no physical signs except slight edema in legs

Coagulation time (2 c c) Mrs N, 20 to 22 minutes
Normal Dr D, 40 to 43 minutes

PROTHROMBIN TEST

Five drops plasma + 2 drops $CaCl_2$ (½ per cent) = clot in nine minutes
Five drops plasma + 3 drops $CaCl_2$ (½ per cent) = clot in twelve minutes

ANTITHROMBIN TEST

This test was made with three dilutions of a new stock of thrombin In two of these the concentration was too great, so that the tubes clotted more rapidly than was convenient for a decisive comparison In each case, however, the clotting in the tubes containing the heated plasma from the patient preceded that in the controls indicating, therefore, less antithrombin in the patient's blood This difference was shown most distinctly with the third dilution of thrombin used as follows

Heated Plasma Drops	Thrombin Drops	Time Interval Minutes	Fibrinogen Drops	Coagulation Minutes
		Normal		
1	3	15	10	15 to 20
		Patient		
1	4	15	10	15 to 20
1	3	15	10	7
1	4	15	10	5

CASE 7.—In a fourth case of thrombosis examined the results were negative. This patient was a hospital nurse with a history of repeated attacks, diagnosed by Dr. Boggs as due to venous thrombosis. At the time of examination of her blood she had been free from any trouble for several months. The examination made was incomplete, including only the coagulation time, thirty-five minutes, and the prothrombin test, ten minutes. As these times were entirely normal the antithrombin tests were not made, owing to circumstances which could not be controlled. This examination was made May 31. Later in the summer the patient had another severe attack, but was not accessible for examination.

The number of cases reported is small, but the indications from the blood examinations of a diminution in antithrombin, especially in Case 2, are sufficiently positive to warrant a suspicion that herein may lie the important factor responsible for the formation of a thrombus. If the antithrombin constitutes a normal defensive agency against intravascular clotting, a diminution in this substance will bring about a condition in which spontaneous coagulation will be favored, particularly in those parts of the circulation in which the mechanical conditions are such as may lead to a partial stasis of the circulation.

In the hemophilic blood there seems to be a relative excess of antithrombin, owing mainly to an actual diminution in the amount of prothrombin. In the blood of patients showing spontaneous thrombosis, just the opposite condition prevails of a deficiency in antithrombin relative to the amount of prothrombin present.

SUMMARY

1. Methods are described for testing the relative amount of prothrombin and antithrombin in the blood.

2. The application of these methods to hemophilia shows that the blood in this condition is deficient in prothrombin. The antithrombin may be normal or somewhat greater than normal. The characteristic peculiarity of hemophilic blood is its markedly delayed time of coagulation. This peculiarity is explained by the diminution in amount of the prothrombin which results in a relative excess of antithrombin.

3. The detection of a hemophilic condition of the blood is facilitated by first oxalating the blood and then recalcifying with an optimum amount of calcium. Under these conditions the time of clotting exhibited by normal plasma is very constant (nine to twelve minutes). That of hemophilic blood is greatly delayed.

4. In patients suffering from spontaneous thrombosis of the veins there is evidence of a diminution in the antithrombin of the blood, the prothrombin being normal. It is suggested that this deficiency in antithrombin operates as a favoring, perhaps as a determining, factor in the production of the thrombus.

5. In purpura hemorrhagica and other forms of so-called purpura, no evidence was found of any variation from normal in either the antithrombin or the prothrombin.

A CLINICAL STUDY OF AMYLASE * IN THE URINE

WITH ESPECIAL REFERENCE TO THE PHENOLSULPHONEPHTHALEIN TEST†

H RAWLE GEYELIN, M D
NEW YORK

Since Majendie,[1] in 1846, first found an amylolytic enzyme in human blood, and later, in 1863, Cohnheim[2] wrote of its presence in the urine, Plósz and Tiegil,[3] Foster,[4] Lépine[5] and many other writers have confirmed these earlier investigators and attempted to determine the quantitative value of amylase with reference to certain pathological conditions, more particularly those of the kidney All the various methods devised were unwieldy and inaccurate however, and most of the information gained contradictory In 1908 Wohlgemuth[6] devised a quantitative test which for the simplicity, accuracy and uniformity of its results so far surpassed any test previously proposed that it has been generally adopted by chemists and clinicians alike for the estimation of amylase in blood, urine, feces and other body substances

Nearly all the contributions to our literature on this subject have come from French and German writers, and in Germany particular attention has been directed to the quantitative estimation of amylase in blood and urine

Wohlgemuth's original method devised for the quantitative estimation of amylase has been followed by numerous investigations into the experimental and clinical aspects of this ferment as found in the body fluids The largest portion of this work has been done by Wohlgemuth and his pupils and has yielded very interesting results

In order that we may have a clearer understanding of what is to follow, it seems advisable to give in detail the technic of Wohlgemuth's

* Throughout this paper the term amylase has been used in preference to the term diastase so generally employed by German writers, amylase having a more restricted significance than diastase, "d" as a means of expressing the readings obtained is used simply in deference to the terminology employed in Wohlgemuth's original method

† From the Blumenthal Fellowship Foundation of the Medical Clinic, College of Physicians and Surgeons, Columbia University

‡Submitted for publication, Aug 1, 1913

1 Wajendie Compt rend, Acad d sc, 1846, xxiii, 189

2 Cohnheim Virchow's Arch f path anat, 1863, xxviii, 241

3 Plósz and Tiegil Arch f d ges Physiol (Pflugers), 1873 vii, 391

4 Foster, M Jour Anat and Physiol, 1867, i, 107

5 Lépine Compt rend, Acad d sc, 1891, cxiii, 1014

6 Wohlgemuth J Biochem Ztschr, 1908, ix 1

"starch-iodin" method for the quantitative estimation of amylase as outlined in his most recent publication on this subject[7] This differs in a few details only from his original method, which may be found in any text-book on physiological chemistry

TECHNIC

Ten test-tubes numbered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 are placed in a test-tube stand (these tubes must be thoroughly cleansed, rinsed in distilled water and carefully dried) To each tube is added 1 c c of a normal saline solution from a graduated pipet (except in Tube 1, which contains 1 c c of the fluid to be tested) With a 1 c c pipet, accurately graduated into 0 1 c c, draw up 1 c c of fluid to be tested and add to Tube 2, thoroughly mixing the fluids by three times drawing up the mixture of normal saline solution and test fluid and again expelling contents into the tube Then finally draw up 1 c c of the fluid now diluted 1 to 2 and place this in Tube 3, repeating the same process of mixing and again withdrawing 1 c c of mixture This is to be repeated in all the succeeding tubes and in the last tube the 1 c c withdrawn can be discarded This will give the following amounts of fluid to be tested in each tube (seven tubes suffice in most cases)

No 1 = 1 0 c c, No 2 = 0 5 c c, No 3 = 0 25 c c, No 4 = 0 125 c c, No 5 = 0 0625 c c, No 6 = 0 032 c c, No 7 = 0 016 c c, No 8 = 0 008 c c, No 9 = 0 004 c c, No 10 = 0 002 c c

Now to each tube are added 2 c c of 0 1 per cent starch solution (Kahlbaum's soluble starch), the tubes fitted into a wire cage after shaking gently, the cage placed on a water-bath at 38 C for half an hour The temperature should be very carefully watched, should be kept within one half degree of 38 C and the time accurately measured At the end of this time all tubes are withdrawn and placed in ice water for five minutes to inhibit further amylolytic activity and are then replaced in test-tube stand To each tube is then added 2 drops of fiftieth-normal iodin solution which must be freshly prepared from a stock solution of tenth-normal strength daily[8] One to one thousand starch solution should be freshly prepared every three days and kept in refrigerator

READING

If the urine is normal the first two or three tubes will be golden yellow in color all starch having been digested, while the fourth will be reddish with no tinge of blue, the fifth violet and the others blue[9] The tube from which the reading is taken is the deep red tube, this tube shows that all starch has been digested at least to the dextrin stage

7 Wohlgemuth, J, and Noguchi, Y Berl klin Wchnschr, 1912, xxiii, 1069

8 In case 2 drops of iodin solution to each tube is not sufficient to bring out a permanent color, add as many more drops to the tube as will be necessary, being careful to add exactly the same number of drops to each of the other tubes

9 The various gradations in color as given above are not always the same, in fact it may be difficult to determine in some instances which tube constitutes the end point or reaction tube but after some practice, the eye becomes trained to recognize that tube which no longer contains a bluish tint Corbett (Quart Jour Med, 1913, vi, 351) in a very recent publication emphasizes the "mauve tube" as the "limit" tube and calculates from the tube preceding this, (the one with a "reddish yellow color") Wohlgemuth (see Note 7) defines the end point as follows "Dasjenige Glaschen, in welchem der erste blaue Farbenton auftritt, das also bei Zusatz von Jod rotblau wird, gilt als Grenze (Limes) der Wirksamkeit und aus der in dem vorhergehenden Glaschen enthaltenen Serummerge wird die Fermentstarke berechnet"

The calculation is then made as follows In Tube 4 there was 0 125 c c urine or fluid which digested 2 c c of a 0 1 per cent starch solution in half an hour at 38 C Therefore 1 c c of fluid to be tested would digest starch according to the following equation

$$0\,125 \quad 2 \text{ c c (starch)} \qquad 1 \text{ c c} \qquad X = 16$$

One c c of fluid digested 16 c c of a 0 1 per cent starch solution in half an hour, this Wohlgemuth designates as follows

$$\frac{38^\circ}{d\text{—}=16}$$
$$30'$$

When twenty-four-hour digestion is employed he expresses it

$$\frac{38^\circ}{D\text{———}=X}$$
$$24 \text{ hr}$$

The present work was undertaken with the object of determining two things first, the clinical value of the estimation of amylase in the urine in determining the functionating powers of the kidney in various diseases, more particularly in renal and cardiac cases, second, the relationship between this test and another test of renal function, namely, the phenolsulphonephthalein test of Rowntree and Geraghty

Most of the cases here reported fall under renal or cardiac headings, as these two conditions are the ones in which functional tests are most often made But several other diseases have also been investigated with very interesting results I had hoped also to make comparisons of the amylolytic value of the blood-serum synchronously with that of the urine, but, except in a few cases, this has not been possible, and will have to be taken up at some future date In all cases investigated, samples of a twenty-four-hour specimen were used for reasons which will be explained later [10]

The quantitative estimation of amylase in the urine as a test for the functional activity of the kidneys has been advanced by Wohlgemuth [11] Benczur,[12] Wynhausen,[13] Rosenthal,[14] Marino,[15] Corbett[8] and Neuman [16] Wohlgemuth[11] used it in conjunction with phloridzin, indigo-carmin and cryoscopy tests to determine unilateral disease of the kidney and found in a series of some fifty cases that it, with the freezing-point test, gave the most valuable results in estimating the relative functionating powers of the two kidneys Wohlgemuth,[7] Corbett,[8] Neuman[16] and Rosenthal[14] have shown that the amount of amylase in the urine of normal individuals

10 A few cubic centimeters of toluol should be added to all twenty-four hour specimens, to prevent bacterial growth This does not interfere with the "d" readings, and preserves the urine for six months or more, giving the same "d" reading at the end of that time as when freshly voided

11 Wohlgemuth, J Ztschr f Urol, 1911, i, 801

12 Benczur, J Wien klin Wchnschr, 1910, xxiv, 890

13 Wynhausen, O J Berl klin Wchnschr 1910, xlvi, 2107

14 Rosenthal, A Deutsch med Wchnschr, 1911, xxxvii, 923

15 Marino, E Deutsch Arch f klin Med, 1911, cm, 325

16 Neuman Deutsch Arch f klin Med, 1913, cix, 164

varied within very narrow limits, and for a given individual was almost constant, provided a twenty-four specimen was used That there may be great variations in the amylolytic power of individual specimens of urine passed at certain times during the twenty-four hours, Wohlgemuth has shown as follows [17] 1 There is more amylase in urine of fasting periods, that is, just before meals 2 The amount begins to diminish after meals and gradually sinks until three or four hours after a meal, then gradually begins to rise again This confirms the earlier investigations of Grutzner,[18] Gehrig,[19] Hoffman,[20] Rosenberg[21] and Leo,[22] who worked with cruder methods

It has also been shown by Wohlgemuth[23] by experiments on dogs that neither the kind nor amount of food taken has any effect on the quantity of amylase in the blood-serum Schlesinger[24] found the same for man, while Corbett[8] concludes that in human beings the quantity of amylase in the twenty-four-hour urine does not vary even with marked dietary changes He gives one instance, however, that of an infant aged 9 months, who had never had unaltered starch food before, and whose urine, after three days' administration of starch-containing food, showed an increase in amylolytic power amounting to almost five times as much as before the giving of starch Neuman[16] also concludes that changes in diet have no effect on the quantity of amylase in urine

The influence[17] of sex on the amount of amylase in the urine shows that the urine of males has a slightly higher "d" reading than that of females This, however, is so small as to be negligible in clinical work

According to Wohlgemuth,[17] Corbett,[8] Wynhauser[13] and Marino,[15] the reaction and salt concentration of the urine has no appreciable effect on the "d" readings In this connection it may be mentioned that Cole,[25] working with a pure (dialyzed) solution of ptyalin, shows that the effect of any electrolyte on the rate of action of the ferment is dependent on two factors — first, the acceleration due to anions other than hydroxylions, and second, the depression due to kations and hydroxylions

Wohlgemuth[26] himself, after careful studies of the salivary amylase, pancreatic amylase and the amylase of blood-serum, all of which he found to have the same properties, came to the following conclusions

1 Sodium chlorid increases amylolytic action in twenty-four hours' digestion ten fold

17 Wohlgemuth Biochem Ztschr , 1909, xxi, 432
18 Grüttzner Breslauer ärztl Ztschr , 1882, xvii, 193
19 Gehrig, J Arch f d ges Physiol (Pflügers), 1885, xxxviii, 38
20 Hoffman Arch f d ges Physiol (Pflugers), 1887, xli, 148
21 Rosenberg Dissertation, Tubingen, 1890
22 Leo Verhandl d VII Kongr f inn Med , 1888, 364
23 Wohlgemuth, J Biochem Ztschr , 1909, xxi, 381
24 Schlesinger, W Deutsch med Wchnschr , 1908, xiv, 593
25 Cole Jour Physiol , 1904, xxx, 202
26 Wohlgemuth, J Biochem Ztschr , 1908, ix, 10

2 The lowest dilution in which sodium chlorid seemed to have an accelerating action was 0 002 to 0 00097 per cent

3 The increased effect of sodium chlorid is due to the anion chlorid, therefore all other chlorid-containing neutral salts accelerate the enzyme

4 Of the other halogen ions bromin also increases the action, also iodin, but to a less degree, while fluorin inhibits

5 Alkalies, except sodium carbonate, inhibit the action of amylase and destroy it by prolonged action

6 Neutral salts like sodium phosphate, oxalate and acetate inhibit the action of amylase, whereas the nitrate, nitrite and chlorate increase the action Sodium sulphate is indifferent

7 Alanin and leucin inhibit, glycocol is indifferent

8 Colloid metals (gold, silver, copper and iron), inhibit in proportionately smaller concentration, while in strong dilution they are indifferent and the addition of sodium chlorid does not alter their behavior

As the amylase in urine is probably derived directly from that of the blood it must have the same properties as that of the blood It can, therefore, readily be seen how difficult it would be to determine the individual factors concerned in the composite action of the various ions on the amylolytic power in such an ion complex as the urine Of the urines I have studied, some two hundred in number, I have had only two and both were from the same patient, which showed an alakine reaction, all the others were neutral or acid, the majority being acid No appreciable effect on the "d" readings caused by reaction could be noted, except when decomposition was evident, this seeming to accelerate the action of amylase

Other factors affecting action of the ferment in urine must here be mentioned First, the action of blood It has been shown[27, 28] that blood and various organ extracts have a marked accelerating action on the amylolytic activity of pancreatic juice, saliva, etc So that urine containing blood will show a much higher amylolytic power than normal urine Dudley Corbett,[8] in a very recent publication has also demonstrated that the influence of blood on the amylolytic action of the urine is a strongly accelerating one Neuman,[16] however, states that in "several cases" during menstruation no effect could be noted He does not state definitely, however, how much blood was present nor does he say whether the specimens examined were complete twenty-four-hour ones Both these factors are important, particularly the former, as I have often centrifuged specimens of urine from menstruating women and in the sediment found only small numbers of red blood-cells, absolutely no macroscopic evidence of blood being present It is only reasonable to

27 Wohlgemuth, J Biochem Ztschr, 1911, xxxiii, 303
28 Pozerski, L Thèse de Paris, 1902, 70

suppose that in these cases when such relatively minute quantities of blood were present, no, or very slight, alteration in the "d" value would be found

Another factor which must be considered in regard to its effect on amylolytic activity in the urine is albumin Very few authors have dealt with this at all Luthje[29] found high amylase value in albuminous urines Rosenthal[14] found that the chronic interstitial forms of nephritis showed uniformly lower readings that did other forms, and generally less albumin is found in the chronic interstitial forms He draws no deductions, however Benczur concludes that high "d" readings are sometimes found in nephritics and other albuminuric conditions He argues that his and Wohlgemuth's experiments[30] in which they found increased "d" value for blood-free kidney extracts of diabetic animals, show that the kidney plays some part in the production of urinary amylase, and further, that the inflammatory condition which produces albuminuria may also serve to stimulate the kidney cells to increased amylase production Neuman[16] denies any influence on the part of albumin or pus Corbett, whose series of cases is small, gives this subject more detailed attention and theorizes as follows The damage of kidney structure may be equally severe in cases with little or much albumin The presence of much albumin is attributed to increased permeability of the kidney and the high "d" values so often obtained in these cases are due to the same factor "For," he argues, "if the renal cells are permeable to the complex protein mixture, generally termed albumin, they should the more readily allow the passage of a simpler colloid, such as amylase" To support this theory he refers to cases of eclampsia with much albumin and many casts in which the "d" values were very high and in which there must have been severe damage to kidney structure All the clinical evidence to date certainly points to higher "d" values in those forms of nephritis with high albumin content of the urine than in those forms with little or no albumin which almost invariably give low readings for "d"

In stating the results in my own series of cases I have adopted a purely arbitrary method in comparing the amounts of albumin found in different cases, for instance, anything tabulated as trace or faint trace is designated as "little albumin," while anything from heavy trace to much albumin or a definite quantitative figure is designated as "much albumin"

On examination of the figures in Table 1 with reference to the influence of albumin on the "d" reading, thirty-four cases[31] are found which have varying degrees of albuminuria, twelve cases which have

29 Luthje, H Festschr f J Rosenthal, 1906, II, 131-138

30 Wohlgemuth, J, and Benczur Biochem Ztschr, 1909, xxi, 460

31 Case 29 is omitted as here the pus was very large in amount and was probably the main source of the albumin

TABLE 1.—CHRONIC INTERSTITIAL NEPHRITIS

Case	Name	Sex	Date	Amylase	Urine				
					Quant c c	Sp Gr	Albumin	Casts	Blood
1	D W	M	10/31/12	8	1,770	1,008	Tr	Many hyal and gran	Occ R B C
			1/15/13	2	1,200	1,015	H Tr		
			3/ 6/13	4	2,020	1 020			
			3/13/13	4	1,500	1 009			
			3/22/13	8	1,200	1 015	H Tr		
2	L K	F	10/31/12	4	850	1 020	H Tr	Many hyal and gran	0
3	E H	F	10/31/12	2	1,230	1 012	V F Tr	Few	0
4	A P	F	10/31/12	8	900	1 018	Much	Waxy gran, hyal	Few R B C
5	J K	M	11/ 3/12	2	1,830	1 027	F Tr	Hyal, gran	Few R B C
6	M R	M	11/ 3/12	4	1,500	1 020	V H Tr	Gran, waxy hyal	Few R B C
7	A W	M	11/27/12	2	1,620	1,008	F Tr	Hyal, gran	0
			12/20/12	0	1,740	1 006			
			2/ 1/13	2	1,560	1 010			
8	G D	M	11/ 2/12	0	930	1 019	V F	Hyal, gran (many)	Few R B C once
			12/16/12	16	1,830	1 013	Tr		
			5/11/13	2	2,040	1 012			
9	S D	F	12/22/12	4	1,400	1 012	F Tr	Occ hyal, gran	Few R B C
			1/ 9/13	4	1,300				
			2/ 1/13	4	1,365				
10	M R	M	1/10/13	2	2,075		V F	0	0
			1/22/13	4	790	1 020	Tr		
			2/ 3/13	2	790	1 030			
			2/14/13	4	1,230				
11	J S	F	1/10/13	4	1,100		V F	0	Occ R B C
			1/16/13	0	1,630	1 012	Tr		
			1/23/13	4	1,280				
			1/26/13	4	730	1 016			
			1/28/13	4	1,450				
12	G H	M	1/15/13	32	1,050		H Tr	Gran, hyal	0
13	F R	M	1/18/13	2	1,200		H Tr	Gran, hyal	0
			2/26/13	2	1,760		F Tr		
14	R P	F	1/18/13	4	1,800		V F Tr (Sugar)	0	R B C few
15	C M	M	1/26/13	2	2,700		H Tr	Many hyal, gran	Many R B C
			1/31/13	2	2,400				
			2/14/13	2	1,400				
16	M S	F	2/ 3/13	8	2,705	1,036	H Tr	Hyal, gran	0
17	J T	M	1/21/13	2	630		V H	Gran hyal	R B C Many for two days after operation
			2/ 4/13	8	995		Tr		
			2/ 6/13	16	750				
			2/12/13	32	630				
			2/13/13	31	660		2%		
			2/15/13	32	1,040				
			2/21/13	32	1,000				
			2/26/13	32	950		V H		
			3/ 6/13	8	1,500		Tr		
			3/13/13	16	930				
			3/17/13	32	700				
			3/19/13	32	900				
			5/ 8/13	32	1,350				

Urine					
Blood Pressure	Reaction	Phenol-sulphone-phthalein Per Cent	Date	Disease	Result
240 (S) to 80 (S) (term)	Acid	55 45 56	10/27/12 11/13/12 3/ 5/13	Chronic interstitial nephritis	Died
170 (S) to 200 (S)	Acid	45	10/26/12	Chronic interstitial nephritis This patient had hyperglycemia and persistent glycosuria Regarded as a case of dys pituitarism	Improved
205 (S)	Acid	26	10/ 3/12	Chronic interstitial nephritis	Improved
130 (S)	Acid	None made		Chronic par neph (Some edema Chronic pul tuberculosis Always fever)	Died
	Acid	F Tr	11/ 2/12	Chronic interstitial nephritis (Atony of bladder)	Improved
130 (S) 80 (S)	Acid	36	11/ 2/12	Had marked amyloid change in kidney, liver and spleen at necropsy	Died
220 (S) 250 (S)	Acid	Less than 5 Trace 1 1	11/22/12 12/ 3/12 1/ 6/13 1/15/13	Chronic interstitial nephritis (Necropsy showed extreme art scl of bl ves, kidney, etc)	Died
180 (S) to 200 (S)	Acid	13	11/ 4/12	Chronic interstitial nephritis (Empyema of frontal sinus, secondary diagnosis)	Died
	Acid	10 5 3	12/ 6/12 1/ 6/13 2/ 3/13	Chronic interstitial nephritis (Secondary anemia)	Improved
Within normal limits	Acid	32 38	1/ 9/13 1/21/13	Chronic interstitial nephritis (Chronic card valv dis)	Improved
200 (S)	Acid	16 10	1/ 9/13 1/24/13	Chronic interstitial nephritis Congenital cystic kidney and hydrothorax	Improved
200 (S)	Acid	33	1/14/13	Chronic interstitial nephritis	Died
220 (S)	Acid	5 Trace	1/16/13 11/25/13	Chronic interstitial nephritis	Improved
170 (S)	Acid	57	1/16/13	Chronic interstitial nephritis (Diabetes not severe but may be primary disease)	Improved
180 (S)	Acid	11 76	1/25/12 2/13/13	Chronic interstitial nephritis (Acute tonsillitis)	Improved
	Acid	48	2/19/13	Chronic interstitial nephritis (Acute cardiac dilatation)	Improved
190 (S)	Acid	40 23 13 16 10 13 31 29 66 60 46 44	10/16/12 11/12/12 12/10/12 12/23/12 1/10/13 1/27/13 2/10/13 2/25/13 4/ 2/13 4/21/13 5/ 6/13 5/24/13	Chronic parenchy nephritis Jan 30, 1913, had double decapsulation of kidneys Since then edema has disappeared Developed symptoms since Feb 19, 1913, of pul tuberculosis with fever Discharged July 8, 1913 Still fever	Improved

TABLE 1.—CHRONIC INTERSTITIAL NEPHRITIS

Case	Name	Sex	Date	Amlase	Urine				
					Quant c c	Sp Gr	Albumin	Casts	Blood
18	M B	F	2/ 6/13	16	1 355	1 016	H Tr	Gran, hyal	0
			2/ 8/13	16	1,200	1 022			
19	E J	F	2/ 4/13	2	1,150	1 010	V H	Very	0
			2/ 5/13	2	1,400		Tr	many waxy	
			2/12/13	0	1,160		0 2%	hyal, gran	
			2/21/13	0	1,000		to		
			3/ 6/13	0	1,005		1%		
			3/13/13	0	900				
			3/16/13	0	520				
20	C W	F	2/ 9/13	2	1,530		H Tr	Hyal	Few R B C
			2/10/13	2	1,470				
			2/21/13	2	1,010				
21	K D	F	2/ 9/13	8	2,100	1 023	V H	Hyal	Much blood
			2/10/13	8	1,120	1 017	Tr	gran	
22	J M	M	2/14/13	16	1,170		V H	Hyal,	0
			2/16/13	16	1,020		Tr	gran	
23	C S	M	2/19/13	8	520	1 025	V H	Hyal,	Many R B C
							Tr	gran	
24	B G	F	2/ 8/13	4	255+		Tr	0	Few R B C
25	N F	M	3/ 6/13	8	1,350	1 010	V H	Hyal,	Few
			3/11/13	0	1 950	1 016	Tr	gran	R B C
			3/12/13	8	1,800		Tr	(many)	
			3/13/13	8	1,260		V H Tr		
			3/17/13	0	1,520				
26	J F G	M	3/ 7/13	8	1,500	1 012	H Tr	Hyal	0
			3/12/13	8	1 450	1 020		gran	
			3/18/13	16	700			(many)	
27	S M	F	3/12/13	0	950		3%	Hyal,	Few
			3/18/13	0	700			gran	R B C
28	P C	M	3/22/13	0	1,170		Tr	Hyal, gran	0
29	C M	M	4/ 9/13	2	1,800	1 010	H Tr	Hyal	R B C much pus
			4/12/13	0	1,870				
			4/16/13	2	2,240	1 022			
30	D C	M	3/ 4/13	4	1,480	1 015	V H	Hyal,	R B C few
							Tr	gran	
31	R C	M	5/14/13	4	900	1 013	H Tr	Hyal, gran	0
32	S C	M	5/ 1/13	4	1,250		Tr	Hyal,	R B C
			5/15/13	4	1,500			gran	few
33	J Y	M	5/22/13	2	1,500		H Tr	Hyal	R B C
			6/15/13	2	1,200			gran	many
34	J M	M	5/15/13	2	1,400	1 015	H Tr	Hyal, gran and fatty	R B C many
35	T V	M	1/25/13	8	905	1 019	Tr	Hyal, gran	0

CHRONIC PARENCHYMATOUS NEPHRITIS—(Continued)

Urine			Date	Disease	Result
Blood Pressure	Reaction	Phenol-sulphone phthalein Per Cent			
210 (S)	Acid	33 69	2/ 7/13 3/10/13	Chronic interstitial nephritis (Chr card valv dis)	
130 (S) 160 (S)	Acid	3 11 10	1/ 6/13 1/27/13 2/ 3/13	Chronic interstitial nephritis Necropsy Chronic dif neph (amyloid) Aortic and mitral endocarditis (Regurgitation and stenosis)	Improved
	Neutral	F Tr F Tr	1/ 3/13 2/14/13	Chronic interstitial nephritis	Unimproved
180 (S)	Neutral	58	2/ 7/13	Chronic interstitial nephritis	Improved
215 (S)	Acid	35	2/13/13	Chronic interstitial nephritis	Improved
200 (S)	Acid	53		Chronic interstitial nephritis Cardiac hypertrophy Tabes dor	Improved
240 (S)	Acid	28 65	2/17/13 3/ 5/13	Necropsy Chronic dif neph Hemorrhage into cerebrum	Died
Above 160 (S)	Acid	12 19	3/ 4/13 3/ 8/13	Chronic interstitial nephritis	Improved
	Acid	44 42	3/10/13 3/22/13	Chronic interstitial nephritis	Improved
	Neutral	None made		Chronic par nephritis	Improved
150 (S)	Acid	13	4/19/13	Acute nephritis, bronchitis	Cured
	Acid	Trace Trace Trace	4/ 8/13 4/15/13 5/12/13	Pyelitis (bilateral) Uremia Delayed excretion urine, bloody, from left ureter	Died
	Acid	28	4/27/13	Chronic parenchymatous neph	Improved
	Acid	15	5/ 9/13	Chronic interstitial nephritis	Died
	Acid	52	3/ 7/13	Chronic interstitial nephritis	Improved
180 (S)	Acid	5 Trace	5/20/13 6/13/13	Chronic interstitial nephritis	Died
	Acid	24 16	5/13/13 6/13/13	Chronic interstitial nephritis (Chr card valv dis)	Died
140 (S)	Acid	70 54	1/13/13 1/23/13	Chronic interstitial nephritis	Died

"little albumin" give subnormal "d" readings, eleven cases which have "much albumin" give subnormal "d" readings, Case 12 showing both, seven cases which have "much albumin" give normal or high normal "d" readings, while two cases in which there was little albumin give low normal "d" readings, and both these cases give normal phthaleins

Cases 1, 17 and 25 do not permit of classification as regards this subject, as all have shown much albumin with varying "d" readings

No hard-and-fast conclusions can be drawn from these figures, both because the method of estimating the quantity of albumin is purely an arbitrary one, and because the number of cases is not sufficient, but the results are at least suggestive of the fact as observed by other investigators that in cases of nephritis with high albumin content there is a greater tendency to normal or high normal "d" values than in those with small albumin content, when the "d" readings are almost invariably subnormal, and further, in those urines, when in spite of a high albumin content, persistently subnormal "d" readings are found, it can be assumed that the functional damage has been all the more severe In all cases we must not forget the possible effect of blood if that be present Also the presence of pus of extra-renal origin as a factor in the production or intensification of the albumin test must not be overlooked, in view of what Neuman[16] asserts, that is, that pus has no influence on the "d" readings [32]

The question of dilution and its effect on the quantitative estimation of amylase is one that has been the subject of much discussion on the part of many observers Wynhausen,[13] Marino,[15] Corbett,[8] Rosenthal[14] and Benczur[12] argue that its influence is a very limited one, and in the range of normal twenty-four-hour urines no appreciable effect is noted on the "d" readings Wynhausen[13] quoted a case in which two readings were identical in the case of a patient who had 700 c c of urine, specific gravity 1 015, and a month later had 2,500 c c of urine, specific gravity 1 015 All the other authors mentioned above quote many similar cases They agree that only in cases of extreme variations in amount do the "d" values change One of my patients who had shown a reading of 32 on two occasions, only five days apart, with urine quantity amounting to 940 c c and 1,300 c c, showed fifteen days later a reading of 8, with a urine quantity of 4,000 c c This was a patient who had an exophthalmic goiter (Case 10, Table 3), whose condition throughout her stay changed very little In each instance the "d" reading was within normal limits, although the effect of the diuresis on the last "d" reading can readily be seen Extremely low amounts of urine (under 500) are found only, as

32 Neuman's work appeared after this investigation had been concluded and the tables made out Allusions to his work have been included only as they could be added to the text and had reference to what has already been written His work on the effect of dilution on the "d" reading has not been alluded to as no time to study its application has been available

a rule, in renal and cardiac condition, and the renal cases in spite of this show a low "d" reading If in a case of this kind the reading should be 8, for instance, in view of the concentration of the urine we would be justified in assuming that were the quantity of the urine within normal limits, the reading would be lower The effect of concentration on the "d" value may be noted in Case 7, Table 2 Here we have an approximately normal excretion of phenolsulphonephthalein and a high normal "d" reading, 32 on three occasions It is probable that urine within normal limits in this case would have brought the reading to a lower "d" worth In general, it may be assumed that only when a urine is abnormally large or abnormally small in quantity allowance need be made for dilution or concentration in judging the value of the actual "d" reading

Rosenthal[14] has made an attempt to estimate what he terms the "absolute diastatic strength" of the urine This he does by multiplying the twenty-four-hour total by the "d" reading and then dividing by 2, for example, daily output 1,500 c c, 0 2 c c of urine digests 2 c c 0 1 per cent starch solution, therefore the absolute diastatic strength =

$$\frac{1{,}500 \times 10}{2} = 7{,}500, \text{ or 500 per cent concentration}$$

I do not see that anything is gained by this method, and as Corbett aptly comments, "the results would vary out of all proportion to slight changes in the daily excretion," which in Rosenthal's series they do

Another factor to be considered is the influence of fever on the "d" values, and here all observers agree that very high readings are found in acute infectious diseases Rosenthal[14] demonstrates the influence of high temperatures in a case of nephritis which developed lobar-pneumonia During the febrile period amylase was present in normal amounts, after subsidence of the fever no amylase could be found in the urine, while the clinical picture was at all times that of nephritis

Having discussed the various conditions which may affect the action of amylase in the urine and having seen that in normal individuals in a twenty-four-hour specimen the "d" readings vary within narrow limits, it is appropriate at this point to summarize briefly actual figures obtained for the "d" values of normal twenty-four-hour urine specimens Wohlgemuth,[7] in a large series of normal cases, says it varies between

$$d\frac{38^\circ}{30'} = 16 \text{ to } 32$$

never exceeding 64 Corbett,[8] working with slightly different dilutions, gives 6 6 to 33 3 with an average of from 10 to 24 Rosenthal,[14] whose method of calculating his readings is different, gives 10 to 25 when his results are translated into terms of the original Wohlgemuth method

From repeated examination of eight normal cases and many other cases whose clinical condition gave no reason to suspect altered "d" readings, I have reached the conclusion that "d" = 8 to 32 is the normal range for twenty-four-hour urine The large majority of the readings were between 16 and 32, as given by Wohlgemuth I have never had a normal urine in which a reading of 64 has been reached

Coming to the study of altered amylolytic power of the urine observations in the literature, so far it has been confined mainly to four conditions, that is, nephritis, diabetes, pancreatic disease and the so-called diabetes insipidus Many other conditions have also been investigated, but the most positive and important results have been obtained in these four conditions

NEPHRITIS

This includes all forms of the disease ranging from the acute type to the chronic (tubular, vascular or mixed) types, and includes also such conditions as pyelitis and renal calculus Hirata,[33] working under Wohlgemuth's direction produced sublimate, chromate and uranium nephritis in rabbits, estimating the amylolytic power of their urine before and after the production of nephritis He found that the "d" values in all forms of nephritis were markedly decreased with a coincident rise on the "d" value of the blood, which was not always as marked as that shown in the urine In view of the fact that these forms of experimental nephritis interfere mainly with tubular function, it would seem probable that amylase was excreted in largest quantities via this route

This work on animals has been further substantiated by the investigations on patients suffering from various forms of nephritis Wohlgemuth,[17] Wynhausen,[13] Marino,[15] Rosenthal,[14] and more recently Corbet[8] and Neuman[16] give distinctly low "d" readings for nephritis in general Benczur[12] does not agree with the above-mentioned investigators, although he admits low readings in some of his cases of nephritis He reports only eleven cases and five of these showed low "d" values Two of the other cases showed very high readings Benczur comes to the conclusion that no reliable information can be drawn from the test It is interesting to note in this connection that although Benczur could not find any "essential" difference between $d\frac{37^\circ}{24\text{ hr}}$ readings of individual specimens and those of twenty-four-hour specimens his five cases of nephritis with low readings were all twenty-four-hour specimens, while the other specimens examined, taken in hunger periods, when as Wohlgemuth shows, the "d" readings are highest, all give much higher amylase values

Certainly the preponderance of evidence so far adduced demonstrates that in the large majority of so-called nephritides the amylase value of

33 Hirata, G Biochem Ztschr, 1910, xxviii, 23

twenty-four-hour specimens of urine is markedly below normal and in very few of the remaining cases does it exceed low normal limits It must be admitted, on the other hand, that undoubted cases of severe chronic diffuse nephritis, proved by necropsy, and by the presence of every characteristic clinical symptom, have shown normal "d" readings, but these cases may also show one of the other functional tests to be normal

In all of the thirty-five cases of renal disease shown in Table 1, the "d" value of the urine has been estimated one or more times In all but two of the cases the phenolsulphonephthalein test of Rowntree and Geraghty has been made, and in the majority of instances on the day preceding the "d" estimation

A word as to the technic of performing the phenolsulphonephthalein test in the First and Second Medical Divisions of the Presbyterian Hospital may be of service The patient is given 200 to 300 c c of water twenty minutes previous to the administration of 6 mg of the drug, which is given intramuscularly in all but a few instances, when it is given intravenously The time of first appearance is noted The specimen for this can usually be obtained by getting patient to void, if this is not possible, a catheter is used Then the urines collected the first and second hours are estimated separately The figures given are the totals of the first- and second-hour amounts The time of appearance and the separate amounts for each hour are not deemed of sufficient importance in their relation to the "d" values to be tabulated With the estimation of amylase, the quantity, specific gravity, reaction, albumin, casts and blood were noted in all twenty-four-hour specimens, not only on day of "d" estimation, but at intervals during their stay in the hospital Albumin and casts are tabulated as an average finding in several examinations during the test periods or albumin as a separate finding when its relation to the "d" reading is important

The diagnoses appended are clinical and made at the time of discharge of patient Secondary conditions and necropsy findings are given when they occur

From Table 1, if we consider eight as low normal limit, it will be seen that twenty-three of the thirty-five cases of nephritis, or 65 7 per cent, show persistent subnormal or absent "d" values If we compare these values with the phenolsulphonephthalein readings we find that twenty of the corresponding cases also show a lowered phthalein excretion, assuming anything below fifty to be subnormal [34] Of the remaining three cases, one (27) was a severe case of nephritis and the phenolsulphonephthalein test was not made, as it was not deemed advisable to

34 Cases 15 and 24 are here included as the later high readings for phenolsulphonephthalein were not explained clinically

disturb the patient, while in the other two cases (14 and 32), which showed phenolsulphonephthaleins of 57 per cent and 52 per cent, respectively, with "d" readings of four in each case, no definite theory can be advanced to explain the discrepancy, unless, as in Case 14, the mild degree of diabetes and secondary anemia can explain it Low "d" readings in these conditions having been found by other observers, Marino,[15] Neuman,[16] Wynhausen,[13] etc Case 32, however, cannot be explained on any such basis

Of the twelve cases which do not show subnormal "d" values in every estimation, three show it in two or more readings and their average would be subnormal Two of these three cases, namely, Cases 8 and 25, show low phenolsulphonephthaleins, whereas the other case (1) shows one lowered phthalein and two readings above normal This latter case was one of severe nephritis and the patient died in a uremic condition

This leaves nine cases of nephritis, as evidenced by clinical signs, in which there was a low normal or normal "d" reading Of these, one (4) did not have the phenolsulphonephthalein test made Of the remaining eight, five, that is, Cases 12 16 18, 22 and 26, gave normal "d" readings and slightly subnormal phthalein readings, while three cases, namely, 21, 23 and 35, gave normal "d" and phenolsulphonephthalein readings

To summarize, then

In thirty-three cases both tests were made

In twenty-two cases subnormal readings were shown for both tests

Three of the remaining eleven show diminished "d" with normal phenolsulphonephthalein (Cases 1, 14 and 32)

Five show diminished phenolsulphonephthalein with normal "d" readings

Three of these cases (16, 18 and 26) show a diminution of phthalein, which was very little below normal, Case 18 having at one time 69 per cent The remaining three agree in that they show normal "d" and phenolsulphonephthalein readings

Case 17, J T, calls for more detailed comment I have included this case among those that give a low reading for both tests, because at the height of his kidney condition they both showed very low readings This was in the latter part of January At that time the edema was extreme, the urine quantities very small, blood-pressure was high and the patient was rapidly going into uremia January 30 a double decapsulation of the kidneys was made, and from that time on until July, when he was discharged, his kidney condition improved remarkably The edema disappeared, the urine increased and the blood-pressure fell Both functional tests came from extremely low readings to high normal readings, one amylase reading, March 6, 1913, showed low normal limit, but immediately rose again The patient developed pulmonary tuberculosis

a month after the operation, and although this improved slightly, it still persisted at the time of his leaving the hospital Three high normal "d" readings were obtained before fever developed, which was persistent but slight

In Cases 1, 8 and 25, we find that there is not absolute agreement in all of the "d" readings, taken as an average, they are below normal, and should be interpreted as such In Case 1 it must be noted that the phthalein average was normal

It is of interest further to note that in every case when the "d" value was normal with small amounts of albumin the phenolsulphonephthalein was also normal

In Case 8, it is of interest to note that the reading of 16 was obtained during a period of marked clinical improvement, whereas the other readings were obtained during the reverse condition

As regards the relationship existing between the "d" reading and the severity of the nephritis, I am unable to draw any definite conclusions In this connection, Wynhausen[18] states that the lower the "d" reading the more severe the nephritis Some patients with low and in some cases absent "d" readings, were discharged apparently improved Of course, there has not been sufficient time in some cases to determine what the outcome will be, while in others there is no data as to what may have occurred after the patient left the hospital

On the other hand, three patients with normal or high normal "d" readings have died In one of these cases (No 4) death was probably due to complicating disease Excluding this case, it will be seen that nine of the eleven patients who died had low "d" readings

CARDIAC DISEASE

In Table 2 nineteen cases of cardiac disease are tabulated In some of these at certain times during which the patients stayed in the hospital, there were marked evidences of acute myocardial insufficiency In all of the nineteen cases phenolsulphonephthalein and "d" readings were made, and in seven of the cases two phthalein readings were made, one at the time of acute decompensation and a second one later Six of these cases showed low readings at the time of decompensation and normal readings later This was rather a striking feature of these cases, a fact that has already been pointed out by Rowntree, Geraghty and Fitz [35] who produced varying grades of chronic passive congestion of the kidneys in dogs, and found in the severe types of congestion a marked diminution in the phthalein output This, Rowntree and Fitz[36] later confirmed in their clinical findings in a series of cardiac cases, which at the time of acute

35 Rowntree, Geraghty and Fitz THE ARCHIVES INT MED, 1913, xi, 121
36 Rowntree and Fitz THE ARCHIVES INT MED, 1913, xi, 258

TABLE 2—CHRONIC CARDIO-VALVUL

Case	Name	Sex	Date	Amylase	Urine				
					Amt c c	Sp Gr	Albumin	Casts	Blood
1	M F	F	10/31/13	16	1,620	1 015	0	0	
2	F P	M	1/ 8/13	128	350+	1 025	H Tr	Hyal, gran	Much
3	J F	M	1/10/13	16	1,470	1 030	Trace	Hyal, gran	Few R B C
4	J C	M	1/13/13	8	2,400	1 020	0	0	0
5	F C	M	1/12/13	16	1,730	1 025	H Tr	Hyal, gran	0
			1/15/13	32	1,290				
6	J H	M	1/16/13	16	1,530	1 015	F Tr	Hyal	0
7	F R	M	1/18/13	32	520	1 030	V F	0	0
			1/31/13	32	300		Trace		
			2/ 6/13	32	450				
8	S C	F	1/22/13	32	605	1 029	V H	Hyal, gran	R B C
			2/17/13	64	1 105		Trace		few
							F Tr		
9	F S	F	1/25/13	16	680	1 017	F Tr	0	0
10	R	M	2/ 5/13	2	1,150		Trace	0	0*
11	J V	M	2/ 4/13	8	3,210	1 030	H Tr	0	0
12	S P	F	2/12/13	32	450		V F	Hyal	0
			2/14/13	16	1,025		Trace		
13	L D	F	2/16/13	16	910		V F	0	0
							Trace		
14	D C	M	2/17/13	32	1,745		F Tr	Hyal	0
15	P D L	M	2/18/13	16	880		H Tr	Hyal, gran	Many R B C
			3/11/13	16	1,700				
16	D	F	3/ 2/13	8	1,000		V F	Few hyal	0
			3/13/13	8	930		Trace		
17	M F	F	4/ 8/13	16	1,350	1 020	F Tr	Hyal, gran	Occ R B C
			4/ 9/13	16	1,400				
18	A W	F	4/ 9/13	16	970	1 025	F Tr	0	0
19	K W	F	1/15/13	16	440	1 015	H Tr	Coarsely gran, hyal	Mod R B C

* Blood-pressure systolic, 110

Urine		Diagnosis	Result
e- in t	Date		
	10/18/12 11/ 5/12 11/11/12 12/ 6/12	Chronic cardio valvular disease (This was during an acute attack of decompensation)	Improved
	1/ 7/13	Chronic cardiac hypertrophy Cardiac dilatation Emphysema of lungs	Died
	1/ 8/13	Chronic cardio valvular disease	Died
	1/11/13	Chronic cardio valvular disease	Improved
	12/19/12 12/23/12	Chronic cardiac insufficiency and myocarditis	Improved
	12/30/12 1/14/13	Chronic cardio valvular disease, aortitis	Improved
	1/16/13	Chronic cardio-valvular disease, mitral regurgitation, aortic regurgitation (Necropsy diagnosis same as clinical)	Died
	1/20/13 2/15/13	Chronic cardiac insufficiency, hydrothorax	Improved
	1/23/13	Chronic cardio-valvular disease, acute endocarditis (fever)	Died
	2/ 2/13	Chronic cardio valvular disease, subacute articular rheumatism	Improved
	2/ 3/13	Chronic cardio valvular disease, auricular fibrillation aortic roughening and stenosis	Improved
	2/ 2/13 2/10/13	Obesity and fatty heart	Improved
	2/14/13	Chronic cardio valvular disease, cardiac hypertrophy and dilatation	Improved
	2/16/13	Chronic cardio valvular disease, septicemia (fever), acute pericarditis	Died
	2/17/13 3/ 7/13	Chronic cardio valvular disease, cardiac hypertrophy and dilatation	Improved
	3/ 1/13 3/12/13	Chronic cardio valvular disease	Improved
	4/ 7/13	Chronic cardio valvular disease	Improved
	4/ 7/13	Chronic cardio valvular disease, hydrothorax	Improved
ar fter our	1/17/13	Chronic cardio valvular disease	Died

insufficiency showed low phthalein readings, with subsequent normal readings when the symptoms of decompensation cleared up and there was no concomitant nephritis

In only one case in Table 2 have synchronous "d" and phenolsulphonephthalein readings been made during the time of decompensation, and later when compensation was restored In this case (No 8), when the phthalein was 4 per cent the "d" value was 32, while nearly a month later the phthalein had increased to 43 per cent and the "d' reading to 64 In another case (No 19), during decompensation, when the phthalein was low 34 per cent, the "d reading was 16, or normal

In view of the fact that at the time of acute decompensation with diminished phenolsulphonephthalein excretion there is nearly always a large amount of albumin and many red blood-cells, we would not expect to find low "d" readings, and in the few cases observed this has proved to be the case, in fact, it has been rather high normal So that we must conclude that the "d" reading shows very little if anything as regards functional activity during the periods of decompensation If it can be shown that during the period of passive congestion, the urine, in spite of the presence of albumin and blood, gives a reading of 8 or 16, and then later when these two constituents are diminished or absent and the heart condition has improved, the reading is still the same or perhaps increased to 32, we might readily assume that what in other circumstances would be taken for a normal reading had really been an expression of impaired function

Generally speaking, twelve out of nineteen cases show normal phenolsulphonephthalein (after the period of decompensation readings of 48 per cent and 47 per cent included) and also normal "d' readings Of the remaining seven cases, three (3, 8 and 11) showed only slightly impaired phthaleins with normal "d" readings and in none of these cases were further phenolsulphonephthalein tests made

Three cases showed phthaleins ranging from 28 to 34 and normal "d" readings Of these last three cases, Case 2 showed very high "d," 128, but there was much blood in the specimen Case 19 showed marked passive congestion of lungs and liver as well as kidneys during the stay in the hospital, and while the "d" reading is 16, the possible influence of concentration of the urine, blood and albumin must be noted The third case in this group is one where the phenolsulphonephthalein was 28 per cent and the "d" reading 16 Here there is a possible influence exerted by fever

There is one atypical case in this group and that is Case 10 Phthalein reads 51 per cent and "d" 2 That there was any renal involvement in this case was certainly not demonstrable clinically, either at the time of admission to the hospital or at any subsequent time It must remain unexplained

In this series of cardiac cases there are only three in which the influence of urinary concentration should be considered, namely Cases 7 and 19 In both these cases we may consider a lower reading than that given to be possible, but the question as to what the "d" readings might have been, had the urine quantities been larger, was not determinable in either case In Case 11, with 3,210 c c of urine and a reading of 8, a higher reading, let us assume, to 16 would still have been in accord with what other cases of this series show

In Table 3 thirteen cases of varied clinical conditions are tabulated In this table each case or each group of cases must be considered separately

DIABETES

First of all let us consider diabetes Only two cases are to be found in this table, while the other two are found in Table 1 It must be acknowledged that both cases in Table 1 cannot be considered as pure cases of diabetes There was doubt in the minds of those who studied Case 14, R P (Table 1), as to whether the nephritis or glycosuric condition was the primary one Also in Case 2, L K (Table 1), the question not only of nephritis but of dyspituitarism entered into the consideration The glycosuria in this case was small but persistent, and failed to yield at all to dietary measures We find that both these cases showed subnormal "d" readings with normal amounts of urine The phthalein in one case, that of L K (Case 2, Table 1), was a shade under the normal, while with R P (Case 14) it was normal The clinical findings all pointed to an underlying chronic nephritis, and for this reason I have classed them as nephritics

Case 6, J C , and Case 12, Wm McG (Table 3), were quite different, as the patients undoubtedly suffered from a primary diabetes mellitus, with large sugar outputs, and in Case 6 multiple furunculosis with diacetic acid and acetone in the urine The urine quantity was low in Case 6, but a portion was lost, in fact, twenty-four-hour specimens were very hard to obtain The patient had a normal "d" reading with little albumin and little blood, as did also Wm McG

No conclusions from these four cases are justified

Foster,[4] as early as 1867, investigated amylase in the urine of diabetics, and concludes that its value does not exceed normal limits Leo[37] and Benderesky[38] found amylase increased in urine of diabetics Lépine[5] found it decreased in the same disease Enriquez and Binet[39] found the amylase values high in diabetes, decreasing as the sugar content of the urine decreased Loeper and Ficar[40] found it decreased, while

37 Leo Verhandl d VII Kongr f inn Med , 1888
38 Benderesky Virchows Arch f path Anat , 1890, 121, 554
39 Enriquez and Binet Compt rend hebd Soc de biol de Paris, 1908, lxv, 577
40 Loeper and Ficar Arch de méd expér et d'anat path , 1907, xix, 722

TABLE 3–

Case	Name	Sex	Date	Amylase	Urine				
					Amt c c	Sp Gr	Albumin	Casts	Blood
1	A N	F	10/22/12	4	Incomplete	1 020 to 1 032	V F Trace	Few hyal	0*
2	J W	M	10/30/12	8	1,590	1 016	V F Trace	Hyal, gran	0
3	C F	F	12/ 4/12	64	800		V F Trace	0	0
4	M G	F	12/ 3/12	32	1,600		0	0	Many pus cells Alkaline (reaction)
			12/ 6/12	32	1 450				alkaline
5	C W	F	12/ 4/12	32	840	1 020	0	0	0
6	J C	F	12/13/12	16	360+	1 024	Trace	Gran, hyal	Sugar Few R B C
7	T M 7years	M	1/15/13	2	830+	1 010	0	0	0
8	T T	M	1/21/13	32	2 500	1 010	F Tr	Hyal, gran, epith	Bile
9	A D	F	1/22/13	8	1,545	1 020	F Tr	Hyal, gran	Few R B C
10	D M	F	1/25/13	32	1 300	1 015	V F Trace	Occ hyal	0
			1/30/13	32	940				
			2/25/13	8	4 000				
11	E B	F	2/28/13	8	550+	1 025	V F Trace	Hyal, gran	Very few R B C
12	Wm Mc C	M	3/12/13	16	1,300	1 040	Trace	0	0
			3/18/13	16	1,300				
13	G K	M	5/30/13	32	465	1 030	V F Trace	Occ gran	0

* Blood pressure 130 systolic

MISCELLANEOUS CASES

Urine		Diagnosis	Result
Phenol-sulphone-phthalein Per Cent	Date		
41 63	10/25/12 10/30/12	Brain tumor (No necropsy)	Died two days after bilateral decompression
24	10/26/12	Multiple myelomata (Bence Jones bodies in urine) Fractured ribs	Improved
None made		Syph arthritis knee	Improved
None made		Pyelitis Cystitis of pregnancy	Improved
None made		Exophthalmic goiter, glycosuria	Improved
None made		Diabetes mellitus, multiple staphylococcic abscesses (fever)	Improved
None made		Diabetes insipidus	Improved
None made		Hypertrophic cirrhosis of liver, cholangitis, ascites	Improved
58	1/21/13	Complete abortion, albuminuria of pregnancy	Improved
69	2/12/13	Exophthalmic goiter	Improved
None made		Asthma	Improved
None made		Diabetes mellitus, syphilis	Improved
47	5/26/13	Chronic bronchitis	Improved

Clark[41] found it absent These were the most important contributions to the literature on this subject prior to 1908, when Wohlgemuth proposed the "starch iodin" method All investigations prior to this had lacked reliable and uniform methods of estimating amylase, consequently these results are questionable

Wohlgemuth[17] with four cases, Wynhausen[13] with forty, Rosenthal[14] with ten and Marino[15] with ten, find generally low readings in diabetes some cases showing entire absence of amylase Corbett[8] confirms this Benczur[12] denies any variation from normal None of the preceding writers seem to appreciate the possible effect of the tremendous diuresis which usually accompanies this disease, as a factor in lowering the "d" reading, or if they do, they merely add that there seems to be no relationship Corbett[8] does, but in spite of this, insists on subnormal readings for the majority of cases under treatment Wynhausen[13] says that the lower the "d" reading the more severe the disease and the higher the reading the milder the disease Another very important complicating factor, which is so often found in diabetes, is nephritis, and this in itself would account for low "d" readings Wynhausen[13] and Rosenthal[14] acknowledge its presence in the large majority of their very low "d" readings, but fail to appreciate its importance Marino[15] also merely alludes to it Even in those cases in which no urinary evidences of nephritis are present, we cannot exclude varying degrees of kidney damage as evidenced, for instance, by decreased permeability for sugar itself, increased blood-pressure, etc

In view of the great importance of these two factors, namely, extreme diuresis and nephritis, on the values of the "d" readings apart from any other condition, it does not seem justifiable in the light of our present knowledge to attribute the low "d" readings or even the absence of amylase to diabetes *per se*, but rather to regard them as evidences of a complicating condition

Case 2, L K and Case 14 R P (Table 1), with low "d" readings, are certainly suggestive of the above, particularly when contrasted with Case 6, J C and Case 12, Wm McG (Table 3), who, without evidence of renal disease, give normal "d" readings

Diabetes Insipidus—Case 7, T M (Table 3), a child aged 7, is the only case of the kind that I encountered Strauss[42] reported two cases in which not only was there no amylase found, but also no pepsin The absence of the latter had only been met twice before, once in a case of uremia and the other in a case of carcinoma of the stomach Rosenthal[43] reports a case in which he also failed to find amylase in the urine, except

41 Clark Glasgow Med Jour 1905, June, 632
42 Straus H Deutsch med Wchnschr 1912, iv, 163
43 Rosenthal A Berl klin Wchnschr, 1912, xlix, 1265

in an occasional examination, when it was greatly diminished Benczur[42] reports one case in which it was present in normal amount Braunschweig,[44] working in Straus' clinic, has also failed to find pepsin in the urine of such patients The case (No 7) reported in Table 3 shows the amylolytic ferment to be greatly diminished

M G (Case 4, Table 3) was not included under the nephritics, as the condition was confined to the right kidney alone, and at the time of these examinations there was no definite evidence of kidney involvement under cystoscopic examination This evidence was present one month later

A N (Case 1, Table 3) shows a possibility of some renal involvement but aside from a very faint trace of albumin and a few hyaline casts, there was no definite evidence from a clinical standpoint It must be regarded as an unexplained case of low "d" reading The patient died two days after bilateral decompression, no tumor was found, there was no necropsy

T T (Case 8, Table 3) had bile in the urine, and is interesting from the standpoint of what Wohlgemuth[45] has shown, namely, that bile in small amounts accelerates amylolytic action, while in large amounts it inhibits the action

It is to be regretted that no cases of pancreatic disease have been studied in view of the tremendously high "d" readings so uniformly found in these cases

CONCLUSIONS

1 The amount of amylase in the twenty-four-hour specimen of a normal individual estimated by the Wohlgemuth method varies within very narrow limits, and for a given case is almost a constant factor

2 The "d" value of the urine, in the vast majority of cases of chronic or acute nephritis, is subnormal or totally absent In the preceding series of thirty-five cases, 77 1 per cent show this

3 Only in extreme variations in concentration under 500 and over 2,500 c c can this factor be considered to have appreciable influence on the "d" reading, and under these circumstances due allowance should be made in the interpretation of values of "d" obtained

4 The majority of normal readings obtained in cases of chronic nephritis were from urines containing considerable albumin, seven out of nine cases with normal readings This simply confirms the work of other investigators and leads us to assume that the presence of albumin itself increases enzymic activity or in the process of its excretion causes more enzyme to be given to the urine Allowance must always be made for this factor in estimating the "d" value in nephritic urine

44 Braunschweig Dissertation, Leipsic, 1910
45 Wohlgemuth, J Biochem Ztschr, 1909, xxi, 447

5 As a test for renal function in nephritis the readings for "d" agree remarkably well with those of the phthalein test, if we adopt a definite normal limit for both tests, namely, 50 per cent for the latter and "d" = 8 for the former The adoption of 50 per cent as low normal limit for the phthalein test is one which I think is pretty well accepted by most clinical observers

6 The degrees of impairment as indicated by percentages in phthalein readings and graded dilutions in the "d" estimation do not permit comparison This could hardly be expected

7 Cardiac cases with symptoms of decompensation do not show lowered "d" values for urine, while the phthalein test is usually subnormal The normal "d" reading in these cases is probably due to albumin or blood or both

8 Final conclusions as to the relative merits of these two tests both as to the diagnosis and prognosis, must await the results obtained from a larger number of cases Meanwhile I would urge that the "d" test be given an exhaustive trial with relation not only to the phenolsulphonephthalein test, but also to other tests of renal function

My cordial thanks are due to Drs Janeway and Longcope for many valuable suggestions, and to the members of the house staff for their cooperation during the course of this work

437 West Fifty-Ninth Street

THE CLINICAL STUDY OF HYPERCHOLESTERINEMIA

HARRY B SCHMIDT, M D
ANN ARBOR, MICH

That lipoidal substances play an important rôle in the animal body is now generally recognized They occur in practically all the body-tissues and the amounts are increased in sclerotic blood-vessels,[1] in the white plaques of retinitis,[2] in xanthomata, in old infarcts, in caseous tuberculous material and in the cholesteatomatous tumors of the ear and cranial cavity The activity of certain anesthetics varies with their lipoidal solubility and hemolytic processes may be accelerated or retarded by the presence of lipoids Quite recently a number of relatively simple methods have been described for determining the amounts of lipoids, and especially of cholesterin, in small quantities of blood or blood-serum As a result, data concerning the quantity of cholesterin in normal and pathological bloods are now rapidly accumulating It is the purpose of the present paper to review these recent studies of cholesterinemia and to present a series of personal observations

The methods used for determining the amount of cholesterin in the blood depend on a variety of principles Since free cholesterin protects red corpuscles from hemolysis by saponin, it has been proposed to estimate the amount of free cholesterin in the blood-serum by determining the degree to which the serum will protect washed red cells from hemolysis by saponin [3] In most other methods the cholesterin is first extracted from the blood or serum and the amounts extracted are then determined either by direct weighing, by weighing after precipitation with digitonin, or by colorimetric methods The extraction is of great importance because cholesterin is present in the blood largely in combination with fatty acids or other substances and the amount extracted depends on the degree to which these combinations are broken up For this reason the simple extraction of dried blood or blood-serum in the Soxhlet apparatus

* From the Department of Internal Medicine, University of Michigan, Department of Medicine and Surgery

* Submitted for publication, July 30, 1913

1 Windaus Ueber den Gehalt normalen und atheromatosen Aorten an Cholesterin und Cholesterinester, Ztschr f physiol Chem , 1910, lxvii, 174

2 Chauffard, A , de Font-Réaulx and Laroche, G Nature cholestérinique des plaques blanches rétiniennes dans un cas de rétinite albuminurique, Compt rend Soc de Biol , 1912, lxxiii, 283

3 Boidin L , and Flandin, C Procédé rapide de diagnostic de l'hypercholestérinémie à l'aide de la saponine, Compt rend Soc de Biol , 1912, lxxii, 28

uniformly fails to yield the total free and combined cholesterin present The method which we have used is based on the methods described by Grigaut[4] and by Weston and Kent[5] In extracting we followed the method of Grigaut, in which a preliminary treatment of the blood-serum with an alkaline alcoholic solution at a high temperature is used to break up the cholesterin compounds The colorimetric estimation of the amounts of cholesterin subsequently extracted was made according to the modification of Salkowski's test described by Weston and Kent

TECHNIC

In brief, our procedures were as follows

Two c c of blood serum were placed in a pressure bottle of 150 c c capacity To this was added 20 c c of 1 per cent solution of potassium hydroxid dissolved in 50 per cent alcohol The cover was screwed down and the whole placed in a boiling water-bath for from fifteen to twenty-minutes After removal from the water bath, the contents of the pressure bottle were allowed to cool and were then shaken with 50 c c of ether The ether was decanted into a separating funnel and 30 c c more of ether was added to the remaining fluid and shaken vigorously as before This was again decanted into the funnel and any aqueous liquid which separated from the ether was removed This was then washed with 80 c c of distilled water The ethereal extract, after separation from the wash water, was poured into an evaporating dish and evaporated nearly to dryness, leaving behind the small yellow oily droplets containing the cholesterin Five c c of chloroform (Merck's blue label) was then placed on the evaporating dish and rotated carefully so as to dissolve the oily droplets as completely as possible This chloroform solution was transferred to a convenient receptacle, and a second 5 c c of chloroform was then used to wash the evaporating dish and were added to the first 5 c c

The amount of cholesterin in the chloroform solution was determined by comparing its color reaction with those of cholesterin chloroform solutions of known strength These standard solutions were prepared by dissolving Merck's cholesterin in pure chloroform (Merck's blue label) and by preparing a series of dilutions of varying strength so that the amount of cholesterin in 1 c c of chloroform varied from 0 0002 to 0 0004 gm A set of test tubes of uniform bore were arranged in a rack and 1 c c each of the known solutions of cholesterin were added in ascending series, as was also 1 c c of the cholesterin solution prepared from the blood-serum It was often necessary to dilute the latter so that the amount would fall within the scale of the known quantities To each tube was now added 0 1 c c of concentrated sulphuric acid, and the mixture was thoroughly shaken After thirty minutes 1 c c of chloroform was added to each tube and after being placed for fifteen minutes in a dark room, the color of the unknown solution was compared with that of the known This method was found to be exceedingly sensitive and differences in the original fluid of 0 00005 gm per c c could be detected Successive readings from the same serum also gave concordant results, and even after several days' standing, little or no diminution in the amount of the cholesterin in the serum could be discovered

4 Grigaut, A Sur le dosage de la cholestérine dans les tissus, Compt rend Soc de Biol, 1911, lxxi, 441

5 Weston, P, and Kent, G Determination of the Cholesterol Content of Human Serum by the Colorimeteric Method, Jour Med Research, 1912, xxvi 531

THE AMOUNT OF CHOLESTERIN IN NORMAL BLOOD

The amounts of cholesterin in normal blood-serum found by various observers who have used methods similar to the preceding, have been fairly concordant Thus Grigaut placed the normal limit from about 1 2 to 1 8 gm per liter of blood-serum, Weston and Kent[5] found from 1 17 to 2 97 with an average of 1 82 gm , Henes[6] found from 1 4 to 1 8 gm per liter of blood-serum, and Widal, Weill and Laudat[7] found 1 74 and 1 95 in two normal fasting individuals Iscovesco[8] has described a more elaborate extraction method, which apparently yields somewhat larger quantities than the above[9] As will be seen from Table 1, the cholesterin in normal blood-serum as estimated by the method which we used, varied between 1 20 and 1 75 gm per liter Amounts of 2 0 gm per liter or over may be regarded as definitely pathological When those with marked thickening of the peripheral arteries and high blood-pressure were excluded from consideration, the age of the individual did not influence the amount of cholesterin in the serum This is of interest in view of the fact that Wacker[10] observed an increase in the cholesterin content of the reserve fat of aged persons, and Robertson and Burnett[11] have advanced the view that an accumulation of cholesterin in the body may account for the well-known incidence of carcinoma with advancing age

TABLE 1 —CHOLESTERIN IN NORMAL BLOOD SERA

Age	Observations	Amount in Gm per Liter
10 19	1	1 40
20 29	5	1 60 1 27-1 75 1 45 1 50
30 39	3	1 65 1 25 1 35
40 49	1	1 20
50 59	3	1 45 1 55 1 20
60-69	4	1 30-1 20 1 30-1 35

The cholesterin of the blood is not distributed evenly between the plasma or serum on the one hand and the red corpuscles on the other Iscovesco[12] found varying relations between the two Henes[6] found that on the average 54 5 per cent of the blood cholesterin was present in the

6 Henes, E Untersuchungen uber den Cholesteringehalt des menschlichen Blutes bei inneren Erkrankungen, Deutsch Arch klin Med , 1913, cxi, 122

7 Widal, Weill and Laudat La lipémie des brightiques, Semaine méd , 1912, xxxii, 529

8 Iscovesco, H Compt rend Soc de Biol , 1912, lxxii, 257

9 Defaye La cholestérémie, Etudes cliniques, Univ de Bordeaux, No 126, 1911-1912 Quoted by Henes

10 Wacker, L Ztschr f physiol Chem , 1912, lxxx, 383

11 Robertson and Burnett Preliminary Communication on the Part Played by Cholesterol in Determining the Incidence of Carcinoma, Proc Soc Exper Biol and Med , 1913, x, 140

12 Iscovesco, H Les lipoides du sang Dosage comparatifs des lipoides des globules rouges et de sérum, Compt rend Soc de Biol , 1912, lxxii, 985

serum and 45 5 per cent was in the corpuscles, so that when the serum was used for determinations the results were higher than when the whole blood was used Grigaut and L'Huillier[13] obtained similar results in normal individuals, but they found that in pathological conditions the amounts in red blood-corpuscles remained fairly constant, even when the amounts in the blood-serum varied widely For this reason the blood-serum is, as a rule, a more sensitive indicator of pathological changes than is the whole blood, and we have, therefore, made all of our determinations with blood-serum

A number of authors have studied the effect of cholesterin ingestion on the cholesterin content of the blood While some have failed to observe any relation between diet and cholesterinemia or between the amount of cholesterin taken by mouth and the amount in the blood, a number of recent workers have confirmed the opinion of those who have held that the cholesterin of the body is derived, in part at least, from the cholesterin in the food[14] Thus Grigaut and L'Huillier[15] were able to raise the cholesterin content of a dog's blood from 1 8 to 3 0 gm per liter by feeding 1 gm of cholesterin a day, and in another animal the content was raised from 1 8 to 3 8 by feeding 2 gm of cholesterin a day After the feeding was discontinued the amounts of cholesterin in the blood-serum returned to normal rather rapidly Similar observations with similar results have been made on man by Widal, Weill and Laudat, by Henes and by Lemoine and Gérard[16] The cholesterin content of the blood may be raised either by feeding pure cholesterin or by giving a diet rich in this substance

PATHOLOGICAL VARIATIONS IN THE CHOLESTERIN CONTENT OF THE BLOOD

It has long been known that the serum of certain patients is milky instead of being clear These lactescent serums are in general associated with increases in the amounts of lipoid substances in the blood as determined by chemical methods, and in general also with hypercholesterinemia Widal, Weill and Laudat[7] have shown, however, that the lipoids may be increased even though the serum is not milky and that no close parallelism exists between the degree of lactescence and the amounts of lipoids determined chemically

13 Grigaut and L'Huillier Taux comparé de la cholestérine des hématies et du sérum dans le sang normal et pathologique, Compt rend Soc de Biol, 1912, lxxii, 202

14 Doree, Gardner, Fraser, Ellis Origin and Destiny of Cholesterol in the Animal Organism a series of papers in the Proc Royal Soc, 1908 to 1912

15 Grigaut and L'Huillier Hypercholestérinémie d'origine alimentaire chez le chien, Compt rend Soc de Biol, 1912, lxxii, 304

16 Lemoine, G, and Gérard Variations du taux de la cholestérine par rapport à l'alimentation, Bull et mém Soc méd d hôp, 1912, 931

Hypercholesterinemia has been frequently encountered in diabetes Gilgaut and Chauffard mention such an increase frequently Burger and Beumer[17] found an increase in four out of five diabetics, three of whom had milky serums Henes found an increase in two out of seven patients Apert, Pechry and Rouillard[18] were unable to find any definite increase in most of the diabetics whom they examined

Our own observations on patients with diabetes are shown in Table 2 It will be seen that pathological increases of the cholesterin were present in six of ten patients, and that the largest amount occurred in a patient with diabetic coma In two of the patients the cholesterinemia was increased after the sugar had been removed from the urine by placing the patients on a standard Von Noorden carbohydrate-free diet Whether this rise was due to an increased amount of cholesterin in the diet or to an increased mobilization of lipoids already in the body, I am unable to say

TABLE 2—CHOLESTERINEMIA IN DIABETES

Name	Age	Blood-pressure	Sugar in urine	Cholesterin in gm per liter	Remarks
Str	20	120	7 0%	3 2	Severe case
Bri	56	145	4 5%	4 15	Coma Mastoiditis
Lak	39	130	+	2 8	Diabetic gangrene
			0	3 3	
Wil	63	175	+	2 25	Arteriosclerosis
McB	53	135	+	1 7	Mild case
			0	1 9	
Wic	38	130	6 8%	2 8	Severe case
Fee	8	110	++	3	Severe case
Dib	58	135	+	1 3	Very mild case
Bar	40	140	+	1 75	Very mild case
Hil	30	?	+	1 8	Mild case

In jaundice due to an obstruction of the common bile duct there is uniformly present a hypercholesterinemia Chauffard, LaRoche and Gilgaut[19] have ligated the common bile duct in the dog and have found that the amounts of cholesterin in the serum rose from the normal of 1 75 gm to amounts which varied between 2 4 and 5 4 gm per liter These same authors[20] have shown that hypercholesterinemia may also result from a clinical obstruction of the common bile duct, whereas in hemolytic jaundice the amount of cholesterin in the blood-serum is not increased In five patients with obstructive jaundice whom we have

17 Burger, M, and Beumer Zur Lipoidchemie des Blutes, Berl klin Wchnschr, 1913, xx, 112

18 Apert Péchery, and Rouillard Mesure de la cholestérinémie chez les diabétiques, Compt rend Soc de Biol, 1912, lxxii, 822

19 Chauffard, Laroche and Gilgaut Recherches expérimentales sur la cholestérinémie après ligature du cholédoque, Compt rend Soc de Biol, 1913, lxxiv, 1093

20 Chauffard, Laroche and Gilgaut Le taux de la cholestérinémie chez les hépatiques, Compt rend Soc de Biol, lxx, 1911, 20

studied there were definite increases in the amount of cholesterin in the blood It seems evident, therefore, that common duct obstruction will lead to an increased amount of cholesterin in the circulation Whether this is dependent entirely on a resorption of the bile or whether it is due in part to an associated disease of the liver, does not seem to be established at the present time

TABLE 3.—CHOLESTERINEMIA IN JAUNDICE

Name	Cholesterin in gm per liter	Diagnosis
Wo	3 50	Infectious (or catarrhal) jaundice
Gr	2 95	Common duct stone with jaundice
Ch	2 85	Common duct stone with jaundice
Sh	3 80	Malignant obstruction of the common duct
Tri	3 90	Malignant obstruction of the common duct

Hypercholesterinemia occurs with frequency in patients with chronic Bright's disease, high blood-pressure and related conditions [21, 7] I have devoted considerable attention to this group of cases with the hope that some relation might become evident between the hypercholesterinemia and the individual manifestations of cardiorenal disease Our results which are shown in Table 4 have been arranged in the following order the first four patients showed marked thickening of the peripheral arteries, but had no increase in blood-pressure and no albumin in the urine The cholesterinemias in these patients were 2 25, 2 3, 1 80 and 1 95, respectively The next group of patients showed chronic arterial hypertension without albumin in the urine In this group the cholesterinemias were 2 0, 2 37, 1 95, 2 75, 1 85, 1 25, 3 2 and 3 3 gm per liter The third group includes those with chronic hypertension and albumin in the urine, but with good functional tests of the kidneys In this group of patients, the figures were 2 0, 5 3, 3 85, 3 85, 1 25, 2 15, 1 75, 2 1, 2 25, 2 97 The final group includes those who showed more or less renal insufficiency as judged by the phenolsulphonephthalein test and an increase of hypobromite "urea" in the blood They are arranged according to the degree of renal insufficiency, the more severe ones being placed last The cholesterinemias in this group of patients were 3 5, 4 0, 1 35, 1 95, 1 85, 1 6, 1 73, 0 85, 0 8 In the first three of the preceding groups a hypercholesterinemia of moderate grade was encountered in the majority of the patients Some of the lower figures may possibly have been due to an associated edema It is interesting that we should have found an increase in the amount of cholesterin in two of four patients selected on account of a marked thickening of the peripheral arteries who

21 Chauffard, Laroche and Grigaut Le taux de la cholestérinémie au cours des cardiopathies chroniques et des nephrites chroniques, Compt rend Soc de Biol, lxx, 1911, 108

TABLE 4.—CHOLESTERINEMIA IN VASCULAR AND RENAL DISEASE

Age	Blood-pressure	Urine	Peripheral Arteriosclerosis	Cholesterin in gm per liter	Phthalein output	Hypobromite "Urea"
			GROUP I			
59	145	neg	marked	2 25		
49	154	neg	marked	2 30	71 2	25
67	120	neg	marked	1 95		
75	120	neg	marked	1 80		
			GROUP II			
	170	neg	marked	2 00	75 0	22
68	180	neg	marked	2 37		
53	180					
	210	neg	marked	1 95	63 3	31
73	190	neg	marked	2 75		
77	240	neg	marked	1 85		
56	210	neg	marked	1 25	48 8	59
					28 0	
59	210	neg	?	3 2	60 5	65
49	180	neg	neg	3 33	35 9	44
					59	
			GROUP III			
38	210					
	130	Hy	mod	2 0	72	36
43	230	casts				
	230	Alb and casts	slight	5 3	40	48
39	210	Alb and casts	slight	3 85		51
46	212	Alb and casts	marked	3 85	77 5	31
47	230	Alb and casts	marked	1 25	60 0	46
65	205	Alb and casts	marked	2 15		5
58	170	Alb and casts	marked	1 75	42 2	38
52		Alb and casts	marked	2 10	74 7	65
45	235	Alb and casts	Mod	2 25		
44	265	Alb and casts		1 97		
			GROUP IV			
48	172	Alb and casts	Neg	3 5	26 2	34
26	250					
	230	Alb and casts	Neg	4 0	34 0	71
54	190	Alb and casts	Mod	1 35	23 9	53
47	204	Alb and casts	marked	1 95	22 3	80
58	225	Alb and casts	marked	1 85	12	1 55
48	170	Alb and casts	Mod	1 45		
				1 6		
				2 1		
45	245	Alb and casts	marked	1 73	6 8	2 02
40	105	Alb and casts	Mod	85	0	4 39
sia		Alb and casts		08		

showed no evident signs of kidney disease, for Chauffard[22] and Henes[6] found no definite relation between arteriosclerosis and cholesterinemia In patients with high blood-pressure but without albumin in the urine and without other signs of nephritis, hypercholesterinemia is also common Of the first three groups, the hypercholesterinemia is most marked in the third in which the high blood-pressure was associated

22 Chauffard, A Les dépots locaux de cholestérine et leur rapports avec cholestérinémie, Rev de med , 1911, xxxi, 176

with evident renal disease The study of Group IV shows that when there is marked renal insufficiency, as measured by the phthalein test and by the accumulation of hypobromite "urea" in the blood, the cholesterin content of the serum tends to fall and may even become subnormal Similar observations have been made by others,[21] and Widal, Weill and Laudat[7] stated that they were able to follow three patients in whom a fall of the cholesterin in the blood coincided with a rise of the incoagulable nitrogen It seems evident from these observations that a hypercholesterinemia cannot be regarded as an index of renal insufficiency, for it may be present in cases without manifest renal disease, and it usually falls at the time of maximum retention of the urinary products So far as our observations go, it would seem that a hypercholesterinemia may be associated with the degenerative processes in the blood-vessels without manifest renal disease

Chauffard and his school have studied the amounts of cholesterin in the blood during acute and chronic infections, and they have found that whereas in febrile tuberculosis the amount of blood cholesterin is diminished,[23] in typhoid fever it tends to be increased during the later stage of the disease [24] They have also shown that in the later stages of pregnancy there is an increased cholesterinemia [25] I have made no special study of these conditions, but have determined the cholesterin content of the blood in various conditions selected at random Of these I wish to mention especially the case of a young girl with multiple tumors in the subcutaneous tissues which clinically resembled neurofibromas who had 5 63 gm of cholesterin per liter of blood-serum Unfortunately, it was not possible to remove one of the tumors for microscopic examination

THE RELATION OF CHOLESTERINEMIA TO LOCAL CHOLESTERIN DEPOSITS

It has been mentioned that in certain pathological conditions there are abnormal deposits of cholesterin in the body, and the question arises as to the relation which may exist between such deposits and an increased cholesterin content of the blood It is conceivable, for example, that the local deposits may depend entirely on local conditions which either favor a deposit of cholesterin from normal body fluids or which may possibly lead to a local production of cholesterin The cholesterin deposits in infarcted areas and in tuberculous caseous material seem to be due to such local changes, and it has been shown[23] that in tuberculosis the cholesterin of the blood is normal or diminished On the other

23 Chauffard, Richet and Grigaut La cholestérinémie au cours de la tuberculose pulmonaire, Compt rend Soc de Biol, 1911, lxx, 276

24 Chauffard, Laroche and Grigaut Évolution de la cholestérinémie chez les typhiques, Compt rend Soc de Biol, 1911, lxx, 70

25 Chauffard, Laroche and Grigaut Évolution de la cholestérinémie au cours de l'état gravidique et puerpéral, Compt rend Soc de Biol, 1911, lxx, 536

hand, the cholesterin deposits may be regularly associated with such marked degrees of hypercholesterinemia as to favor the view that they result in large part from a surcharging of the blood with this substance In such cases they may well be compared with the tophi of gout and one may speak of a cholesterin diathesis Multiple tuberous xanthomas have been shown to be associated with marked local deposits of cholesterin,[26] and they are known to occur especially in association with general conditions, such as diabetes and jaundice, in which marked degrees of hypercholesterinemia are apt to be present Furthermore, they have been shown to be associated with high degrees of cholesterinemia,[27, 28] so that they seem to depend on a surcharging of the blood with this substance According to Chauffard and Laroche,[29] a similar relation exists for the more common xanthelasmas of the eyelids which are frequently encountered in chronic jaundice, and in two of four cases these authors demonstrated that xanthelasmas were associated with hypercholesterinemia Greater interest attaches itself to the relation which exists between hypercholesterinemia and the cholesterin deposits in the blood-vessels, the retinae and the arcus senilis corneae which are so often associated with diseases of the kidneys and of the arteries We know that hypercholesterinemia is frequent in such patients, and one is tempted to attribute these degenerations in part at least to the hypercholesterinemia present,[30] and especially so as Anitschkou[31] has shown that experimentally one may produce similar changes in the arteries by the administration of cholesterin On the other hand, the cholesterinemia is by no means constant in these patients, and when present it is usually of moderate degree Should one assume, therefore, that the cholesterin deposits were secondary to changes in the blood, one must postulate that there had been times of greater cholesterinemia during which the deposits occurred The proof of such an assumption is not easily furnished and at present it seems more plausible to assume that these cholesterin deposits occurred secondary to degenerative changes in the tissues Nevertheless the frequent occurrence of hypercholesterinemia in such patients and the experimental work of Anitschkou has opened up the whole subject of the relation of arteriosclerosis to hypercholesterinemia and a solution of

26 Pollitzer S, and Wile, U J Xanthoma tuberosum multiplex, Jour Cut Dis, May, 1912

27 Thibierge and Wiessenbach Xanthome tubéreux disséminé et généralisé avec hypercholestérinémie, Bull et mem Soc méd d hôp, 1911, xxxi, 93

28 Legendre, Joltrain and Levy-Franckel Xanthome disséminé et généralisé avec chéloides secondaires, Bull et mem Soc méd d hôp, 1912, xxxiv, 539

29 Chauffard and Laroche Pathogénie du xanthélasma, Semaine méd, 1910, xxx, 241

30 Lemoine, G Théorie de l'artério sclérose basée sur la cholestérinémie, Bull et mém Soc méd d hôp, 1912, xxxiii, 227

31 Anitschkou Die pathologischen Veranderungen innerer Organe bei experimentellen Cholesterinesterverfettung, Deutsch med Wchnschr, 1913, xxxix, 751

this question is all the more urgent for the reason that should there be any relation between hypercholesterinemia and arteriosclerosis it would lead to an endeavor to control the cholesterin content of the blood as a prophylactic measure in the prevention of arterial degeneration

CONCLUSIONS

By the method which we have used, the cholesterin in the blood of normal individuals is found to vary within rather narrow limits It was definitely increased in obstructive jaundice, in six of ten patients with diabetes, and most patients with marked thickening of the peripheral arteries, chronic arterial hypertension and chronic nephritis When, however, there is a marked functional insufficiency of the kidney, the cholesterinemia may fall to normal or even below normal In a patient with multiple subcutaneous tumors (neurofibromas?) there was a marked hypercholesterinemia Excepting in the case of obstructive jaundice, where the cause of the hypercholesterinemia is in part at least due to a resorption of bile, little is known about the etiology of pathological increases in the amount of cholesterin in the blood

THE UREA CONTENT OF THE SPINAL FLUID WITH SPECIAL REFERENCE TO ITS DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE

A SERIES OF NINETY-SEVEN CASES *

WILLARD B SOPER, M D, AND SELMA GRANAT
NEW YORK

In a study of the literature concerning the urea content of the spinal fluid, we have been struck by the amount of work done on this subject by French clinicians as contrasted with its apparent neglect by German, English and American clinicians

The normal quantity of urea in the spinal fluid, as indicated from a review of a considerable mass of material in the literature, varies between 0 01 per cent and 0 05 per cent Mollaid and Froment,[1] in 1909, reviewed the literature up to that time and cited the works which follow in brief Richet, in 1897 gave twenty-five analyses and stated "possibly urea" Deniges and Sabrazès, and Sabrazès and Binaud [2] in 1895, reported two cases of tuberculous meningitis showing 0 015 per cent and 0 035 per cent of urea in the spinal fluid Comba,[3] in 1899, reported two cases of acute uremia, in which there was an increase in the total nitrogen of the spinal fluid Dirksen,[4] in 1900, reported four cases, one of uremia in which the urea content was 0 04 per cent, while three cases in which there was no uremia showed 0 01 to 0 015 per cent Carrière,[5] in 1905, stated that he had found the normal amount of urea to be 0 01 to 0 015 per cent Emery[6] gave the normal as 0 035 to 0 04 per cent, Widal and Froin[7] as varying between 0 015 and 0 035 per cent, Castaigne and Weil[8] from 0 to 0 15 per cent, Graham Forbes[9] as 0 035 to 0 04 per

* From the Laboratory of the Presbyterian Hospital
* Submitted for publication Aug 1, 1913

1 Mollard and Froment Urée dans le liquide cephalorachidien et urémie Nerveuse, Jour de Physiol et de Path Gen, 1909, ii 263

2 Sabrazès and Binaud Traité de pathologie Générale de Bouchard 1897, vi, 650

3 Comba Clin Med Ital, 1899, No 9, p 555

4 Dirksen Etude sur la composition chemique et la concentration moleculaire du liquide céphalorachidien, Thèse de Paris, 1900 to 1901

5 Carrière Etudes sur le liquide céphalorachidien dans l'urémie nerveuse, Séances et memoires de la Soc de Biol, 1905, p 239

6 Emery Clinical Bacteriology and Hematology, Blakiston Sons & Co, 1912

7 Widal et Froin L'urée dans le liquide céphalorachidien de Brightiques, Gaz d hôp, Oct 25, 1904, p 1182

8 Castaigne and Weill Considerations pratiques d'order clinique et therapeutique sur le liquide céphalorachidien des urémiques, Jour méd franç, 1911, v, 34

9 Forbes Pathology of the Cerebrospinal Fluid, Lavori e reviste di chimicae microscopia clinica, October, 1908, p 33

cent Finally, Mollard and Froment asserted that normally the quantity of urea in the spinal fluid was negligible, but in Bright's disease it might increase to a point of diagnostic or prognostic significance From the study of twenty-three cases collected at that time they concluded (a) that a notable increase of urea in the spinal fluid could not be considered as pathognomonic of uremia with involvement of the nervous system, since it might be found whenever the kidneys functionated improperly, (b) that a urea content attaining or exceeding 0 4 per cent meant a pure uremia and foretold a rapidly approaching fatal outcome, (c) that a urea content below 0 4 per cent did not permit of definite conclusions, but should be considered in conjunction with concomitant symptoms

In 1910, Froment[10] reviewed the literature, included the report of cases in his preceding article with Mollard, and collected reports of thirty-four new cases, making in all fifty-seven cases reported up to 1910

From the study of these cases, Froment drew the following conclusions

That all pathological conditions without kidney involvement showed a negligible quantity of urea in the spinal fluid, namely, 0 to 0,015 per cent and that it never attained 0 1 per cent, while in nervous uremia the content varied between 0 15 and 0 45 per cent, that some cases without a definite picture of uremia showed a definite increase of urea, as for instance 0 25 per cent to 0 29 per cent in cases of arteriosclerosis or Bright's disease in which at necropsy there were found multiple cerebral softenings, cerebral hemorrhages or meningitis, that one could determine the association of retained urea and uremia even though other pathological states existed, that with a spinal fluid urea attaining or exceeding 0 4 per cent one could definitely say that a pure, or more rarely, an associated uremia existed and foretell a nearing fatal outcome With less than 0 1 per cent of urea the diagnosis of uremia should be rejected If the urea content of the spinal fluid lay between 0 1 per cent and 0 3 per cent the condition was frequently but not always to be interpreted as a pure uremia while one could not reject all other diagnoses Should there be uremia the prognosis was often favorable

Since our series of ninety-seven cases is practically limited to spinal fluids, we have so far said nothing as to the urea content of blood While the urea content of spinal fluid was being studied and reported that of the blood and body fluids was being concurrently investigated In almost all cases the urea content of all these fluids was found to agree There were occasional discrepancies, but these were largely to be explained by variations in the time of obtaining the fluid and in the methods of examination All observers tend to the conclusion that the quantity of urea is practically the same in all body fluids under similar circumstances In 1906, Javal and Adler[11] reported four cases with determinations of

10 Froment Diagnostic et prognostic de l'urémie nerveuse par le dosage de l'uree dans le liquide céphalorachidien, Lyon méd, 1910, i, cxiv, 269

11 Javal and Adler La diffusion de l'urée dans les transudats de l'organisme Application au diagnostic et au prognostic de l'urémie, Séances et Mém de la Soc de biol, 1906, lxi, 235

the urea content of more than one body fluid, all tending to establish an accord In 1910, Javal and Boyet[12] reported the examination of the body fluids in ten cases with practically an agreement throughout Several authors reported isolated cases in which the urea content of blood and spinal fluid was about the same It remained for Javal[13] to establish this fact This he did as the following study will show In all cases the different fluids were obtained at the same time and the urea content considered as that part of the total nitrogen decomposable by sodium hypobromite

The estimation of urea was made on blood and spinal fluid in eighteen cases, on blood and edema fluid in four cases, on pleural fluid and edema fluid in two cases, on ascitic fluid and edema fluid in one case, on blood and pleural fluid in five cases, on pleural fluid and spinal fluid in three cases In all of these the urea content was practically identical, the variation being negligible, usually about 0 005 per cent, occasionally 0 01 per cent Javal himself regarded the uniformity as established

Accepting this fact, Castaigne and Weill[8] state that inferences may be drawn from examination of the spinal fluid which will be identical with those drawn from examination of the urea content of blood They also report four cases in which uremia terminated fatally and in which the spinal fluid urea varied from 0 115 per cent to 0 767 per cent, and twelve cases in which uremia terminated in recovery, temporary at least, in which the spinal fluid urea was 0 1 per cent to 0 2 per cent Finally, they state that the urea content never reaches 0 1 per cent in normal states while in established uremia it may vary from 0 1 per cent to 0 5 per cent, or even higher

From the work of Widal[14] and further work of Javal[15] on the parallelism in the urea content of different body fluids, the variations in the urea of the spinal fluid assume an importance equal to that of the blood urea Widal states that, with a blood urea content of more than 0 05 per cent, there is some retention, and that one can follow at times the gradual increase from 0 06 per cent to 0 07 per cent, and so on upward He regards a quantity of urea below 0 2 per cent as justifying a prognosis for life of more than one year, a quantity between 0 2 per cent and 0 3

12 Javal and Boyet La Retention de l'uree et sa diffusion dans les liquides de l'organisme, Séances et Mém de la Soc de Biol, 1910, lxviii, 527

13 Javal Contribution Numérique à l'étude de la composition clinique de séreosités physiologiques et pathologiques de l'organisme chez l'homme, Jour de phys et de path gén, 1911, p 508

14 Widal Le prognostic dans le mal le Bright par le dosage de l'urée du sang Les rémissions temporaires et trompeuses de l'azotèmie, Bull et Mem de la Soc de Hôp de Paris, 1911, xxxii, 627

15 Javal Le grande azotémie, ses formes, son evolution son prognostique etudies par le dosage méthodique de l'urée dans le sang et dans les serosités de l'organisme Bull et Mém de la Soc de Hôp de Paris, 1911, xxxii, 485

per cent as indicating a survival of only weeks or months, and a quantity above 0 3 per cent as indicating a not far distant fatal outcome He cites eighteen cases in which the blood content of urea was 0 2 per cent to 0 3 per cent of urea The longest survival was seven months, while thirteen patients died within two months Of thirteen cases with a quantity above 0 3 per cent, all died within one month

Javal, too, agrees with Widal that with a blood urea content above 0 2 per cent the fatal outcome is a question of weeks or months Of his twenty-one cases, the one that survived longest lived but twenty-one months In agreement with Widal he cites cases with marked remissions, but always with an eventual fatal outcome

In the year 1912, several authors reported the urea content of the spinal fluid in infants and children Nobécourt and Darré[16] reported twelve cases in all of which there was evidence of nephritis Of five cases in which there was either simple albuminous nephritis or the type associated with retained chlorids, the urea of the spinal fluid was well within normal limits In four cases conforming to the type of combined urea and chlorid retention, the minimum urea content of the spinal fluid was 0 044 per cent, and the maximum 0 083 per cent In a third class, comprising three cases, the lowest figure on admission to the hospital was 0 123 per cent, and the highest 0 204 per cent These three patients left the hospital greatly improved with the spinal fluid urea within the normal limits The subsequent course of the disease in these patients was not known

Nobécourt, Bidot and Maillet[17] report twelve examinations of the spinal fluid in infants They concluded from these that a urea content above 0 1 per cent is, as a rule, soon followed by death Six infants in their series had over 0 1 per cent and less than 0 2 per cent of urea in the spinal fluid The longest duration of life among the six infants was twenty-nine days The urea content of the spinal fluid in three of the series was above 0 2 per cent, and the longest duration of life among these patients was three days

Nobécourt, Sevestre and Bidot[18] reported fourteen new examinations of the spinal fluid in infants under one year of age Five of these showed urea content of 0 04 per cent to 0 06 per cent, all without apparent kidney lesions, with the exception of one (0 057 per cent urea), which showed at necropsy lesions of the kidney epithelium and a little thicken-

16 Nobécourt and Darré L'urée du liquide céphalorachidien dans les nephrites de l'enfance Bull et mém Soc de hôp de Paris, January, 1912, p 16

17 Nobécourt Bidot and Maillet Valeur prognostique de l'élévation du taux de l'urée dans le liquide céphalorachidienne des nourrissons, Bull et Mém de la Soc de Hôp de Paris, July, 1912, p 47

18 Nobécourt Sevestre and Bidot *L'urée dans le liquide céphalorachidien* des nourrissons, Arch de méd des Enf November, 1912, p 826

TABLE 1.—Pathological Conditions, Nephritis Clinically Excluded

Case No	Age Years	Urea Pct Spinal Fluid	Diagnosis Clinical	Affecting Diagnosis	Further Examination of Spinal Fluid
1	33	0 02	Cerebral tumor Death		Differential Polymorphs, 15%, lymphocytes, 85%
2	11/12	0 05	Tuberculous meningitis		Fluid bloody One examination No tubercle bacilli found
3	3	0 027	Acute anterior poliomyelitis	Paralysis of face, weakness of legs Improvement	Cells per c mm, 5 Butyric acid test neg Polys, 2%, lymphos 98%
4	37	0 053	Acute general tuberculosis		25 c c bloody fluid
5	3	0 044	Tuberculous meningitis		12 c c clear fluid Polymorphs, 3%, lymphos, 71%, endoth, 26%, NaCl, 0 642%
6	3	0 083	Tuberculous meningitis		Lymphocytes, 100% 10 c c clear fluid Tubercle bacilli present
7	3	0 029	Tuberculous meningitis		Tubercle bacilli present
8	11	0 04	Pneumococcus meningitis		Pneumococci present
9	18	0 033	Tuberculous meningitis		Tubercle bacilli present
10	29	0 08	Syphilitic meningitis	Wassermann positive in both blood and spinal fluid	Differential Lymphos, 94% Butyric acid test positive
11	8/12	0 043	Lobar pneumonia with convulsions		Butyric acid test negative NaCl, 0 747% Cultures sterile
12	7/12	0 058	Pneumococcus meningitis	Necropsy No kidney involvement	Pneumococcus present NaCl, 66%
13	13	0 053	Tuberculous meningitis		
14	22	0 053	Suppurative meningitis Mastoiditis		
15	5½	0 04	Streptococcus meningitis Mastoiditis		Streptococcus present
16	2½	0 033	Tuberculous meningitis		Tubercle bacilli present
17	11/12	0 022	Bronchopneumonia Furunculosis		
18	31	0 067	Meningococcus Septicemia, infective endocarditis Necropsy		
19	41	0 033	Tuberculous meningitis		Tubercle bacilli present
20	22	0 049	Otitis media, acute		
21	6	0 036	Suppurative meningitis		
22	6/12	0 035	Tuberculous meningitis		Tubercle bacilli present
23	5	0 022	Catarrhal gastritis	Convulsions, cause not determined	
24	4	0 029	Acute general tuberculosis		No cells
25	35	0 071	Disseminated myelitis	Slight albuminuria Bloood-pressure 110	
26	5	0 026	Tuberculous meningitis		
27	22	0 049	Suppurative otitis media	Albumin, faint trace, no casts	

TABLE 1.—Pathological Conditions, Nephritis Clinically Excluded.—(Continued)

se)	Age Years	Urea Pct Spinal Fluid	Diagnosis Clinical	Affecting Diagnosis	Further Examination of Spinal Fluid
3	15	0 053	Bronchopneumonia	Albumin, faint trace, no casts	Normal
)	2½	0 053	Tuberculous meningitis	Drowsiness to coma	
)	19	0 053	Tuberculous meningitis	Albumin, faint trace, no casts	Tubercle bacilli present
.	6	0 019	Tuberculous meningitis	Stupor	Lymphocytes, 99% N tubercle bacilli found
?	15	0 024	Tuberculous meningitis	No albuminuria No casts	Lymphocytes, 91%
;	34	0 053	Erysipelas	Hysteria	Lymphocytes, 91%
.	53	0 025	Chronic alcoholism Cerebral edema	Delirium tremens No albuminuria No casts	
;	46	0 053	Cerebral glioma	Necropsy Kidneys normal	
;	38	0 026	Tertiary syphilis Gumma of lenticular nucleus	Necropsy Kidneys normal	
		0 053	Tuberculous meningitis Suppurative otitis media	Faint trace albumin No casts	Tubercle bacilli present
;	3¾	0 035	Tuberculous meningitis	Albumin, faint trace, granular casts	Lymphocytes, 67%
)	11	0 014 0 038	Cerebral tumor	Operation Trace albumin No casts	
)	31	0 053	Lobar pneumonia Alcoholic delirium Tubercular meningitis	Trace albumin	Tubercle bacilli present
	7	0 01	Tuberculous meningitis		Tubercle bacilli present
;	36	0 053	Bulbar palsy Tertiary syphilis	No kidney symptoms	
	48	0 059	Suppurative meningitis Suppurative otitis media	No renal symptoms	Pneumococci present
:	16	0 010	Acute tonsillitis Chronic valvular cardiac disease	Trace albumin, few hyaline casts	
;	1	0 038 0 04 0 045 0 07	Chronic basilar meningitis	Necropsy No renal lesion	
;	38	0 135	Acute general tuberculosis Tubercular meningitis	Necropsy showed only a few miliary tubercles of kidney Some congestion of glomeruli and slight degeneration of tubular epithelium	Tubercle bacilli present
	8	0 179	Tuberculous meningitis	Duration two weeks Delirium Coma Fundi show some congestion of discs No albuminuria No casts	Tubercle bacilli present Lymphocytes, 76%
;	53	0 03	Locomotor ataxia		Wassermann positive
)	26	0 018	Chronic pulmonary tuberculosis		

TABLE 1.—PATHOLOGICAL CONDITIONS, NEPHRITIS CLINICALLY EXCLUDED.—(Continued)

Case No	Age Years	Urea Per Spinal Fluid	Diagnosis Clinical	Affecting Diagnosis	Further Examination of Spinal Fluid
50	40	0.115	Paresis	Very slight albuminuria Casts in one of three examinations Blood-pressure 140 Eye-grounds normal No nephritic history, no signs	Wassermann positive
51	2½	0.053	Enterocolitis		
52	12	0.035	Chronic pulmonary tuberculosis	Urine normal	
53	8/12	0.041	Acute general tuberculosis	Urine normal	
54	3½	0.026	Acute general miliary tuberculosis		Tubercle bacilli present
55	18	0.029	Fracture base of skull Suppurative meningitis		
56	21	0.107	Perithelioma of spinal cord	No abnormalities of urine No evidence of nephritis Diagnosis by operation	

ing of the connective tissue. One case showed 0.17 per cent. and 0.23 per cent. of urea in the spinal fluid on successive examinations. At necropsy there was some thickening of the connective tissue of the capsule and its capillaries. The liver, on the other hand, showed marked fatty infiltration and some degeneration. There was one case which exhibited signs of meningitis that were followed by gastro-intestinal disturbances. The last case showed, in two examinations, 0.189 per cent. and 0.377 per cent. of urea in the spinal fluid. Death occurred three days after the second lumbar puncture. No necropsy was made.

With all these facts in mind our own series of ninety-seven examinations has been to us most interesting.

Our method of estimating urea has been as follows. To 120 c.c. of a mixture of 40 parts of ether and 80 of alcohol, all the spinal fluid available was added and allowed to stand twenty-four hours in a glass-stoppered bottle. The mixture was then filtered through smooth filter-papers, the latter being moistened with absolute alcohol. The bottle was then carefully washed and the precipitate cleansed at least four times with hot absolute alcohol. The filtrate was evaporated to a syrupy consistency. The residue was then taken up once more with absolute alcohol, filtered and reevaporated to a very little syrupy liquid. This was taken up in distilled water and put into a small vial in the hypobromite apparatus. One c.c. of nitrogen gas then equalled 0.002694 gm. of urea.

TABLE 2.—Uremia Resulting in Death

Case No	Age Years	Urea Pct Spinal Fluid	Diagnosis Clinical	Affecting Diagnosis	Further Examination of Spinal Fluid
1	47	0 120	Nephritis, chronic Acute uremia Death	Albuminuric retinitis Blood pressure 240 300 mm of mercury No necropsy	15 cc clear fluid No cells
2	45	0 062 Blood 0 07	Chr interstitial neph Acute uremia General arteriosclerosis	Albuminuric retinitis albuminuria, casts, coma No necropsy	No cells Butyric acid test negative
3	32	0 436 Blood 0 365	Chr interstitial neph Acute uremia Death	Albuminuric retinitis Convulsions Blood pressure 198 mm Albuminuria, casts	NaCl 0 802
4	35	0 402 Blood 0 363	Chr interstitial neph Acute uremia	Albuminuric retinitis Blood-presure 180 230 Nausea, vomiting Albuminuria, casts	
5	43	0 571 Blood 0 624	Chr interstitial neph Acute uremia	Albuminuric retinitis Blood-pressure 204 to 220 of mercury Necropsy	No cells
6	25	0 107	Chr interstitial neph Acute uremia General arteriosclerosis	Albuminuria and casts Blood-pressure 170 240 of mercury	
7	65	0 14	Chr interstitial neph Acute uremia	Albuminuria and casts Blood pressure 199 mm	
8	35	0 161	Chr neph Cardiac decompensation	Albuminuria Casts Died cardiac death Blood-presure 148 160	
9	50	0 619	Chr interstitial neph General arteriosclerosis Albuminuric retinitis Uremia	Albuminuria very slight Occasional casts	
10	40	0 311 Blood 0 343	Chr interstitial neph Acute uremia Cardiac decompensation	Blood-pressure 180 mm of mercury	Polymorphs, 99%
11	35	0 402 Blood 0 303	Chr neph Uremia	Albuminuria, casts Nausea, vomiting albuminuric retinitis	
12	42	0 26	Chr interstitial neph Uremia Cardiac decompensation	General edema Stupor Necropsy	
13	26	0 673 Blood 0 28	Chronic diffuse neph Uremia	Prolonged kidney history Semi-coma Anuria	Polymorphs, 92%
14	32	0 026	Chronic diffuse neph Uremia	Kidney history of two years Edema, dyspnea, oliguria, headache, coma Marked albuminuria	
15	63	0 35 Blood 0 32	Chronic diffuse neph	Headache, vomiting oliguria, albuminuria Coma.	

TABLE 2.—UREMIA RESULTING IN DEATH.—(Continued)

Case No	Age Years	Urea Pct Spinal Fluid	Diagnosis Clinical	Affecting Diagnosis	Further Examination of Spinal Fluid
16	31	0 087	Chronic parenchymatous nephritis	Stupor Albuminuric retinitis Marked albuminuria Many casts Blood-pressure 195-225 mm	Polymorphs, 83% NaCl 0 77%
17	27	0 525 Blood 0 538	Chr interstitial neph Acute uremia Cardiac hypertrophy	Blood-pressure 158-192 Marked albuminuria Casts, convulsions, coma	
18	42	0 177	Chr interstitial neph Cardiac hypertrophy Uremia	Headache Systolic blood-pressure 185-235 mm Albuminuric retinitis Stupor	
19	32	0 298 Blood 0 308	Chr interstitial neph Empyema Frontal sinus	Marked albuminuria Many casts Systolic blood pressure 138-195 mm	
20	33	0 33 Blood 0 49	Chr interstitial neph	Albuminuric retinitis	
21	25	0 453 Blood 0 471	Chr interstitial neph	Convulsions Retinal hemorrhages Blood-pressure 178-190 mm	

All the determinations were made by one of us (S G), using always the above method The disagreements in the amounts of blood and spinal fluid urea that are occasionally reported, are probably due to the fact that the determinations were not made at the same time

The cases, as may be seen from the tables, are arranged in four groups (1) cases in which nephritis could be clinically excluded, (2) cases in which death was caused by uremia, (3) cases in which clinical nephritis did not terminate fatally, and (4) pathological conditions with a diagnosis other than uremia, from which, however, nephritis could not be excluded

Of cases belonging to Group 1, in which nephritis could be excluded, there were fifty-six Thirty-three of these cases showed less than 0 05 per cent urea in the spinal fluid, fifteen cases 0 05 to 0 06 per cent, four cases showed 0 067 per cent, 0 071 per cent, 0 08 per cent and 0 083 per cent, respectively, with no satisfactory explanation, and four cases show, respectively, 0 135, 0 179, 0 115 and 0 107 per cent In none of the last four was there sufficient ground to explain the high urea content

In the second group, which comprises twenty-one cases in which uremia was present, the constant high percentage of urea in the spinal fluid was very striking In thirteen cases it was above 0 2 per cent, in

five cases between 0 1 per cent and 0 2 per cent, and in three cases under 0 1 per cent Two of the last three were, however, above normal

In Group 3 there were eight non-fatal cases of nephritis The two patients in this group (Nos 1 and 2), who showed marked increase in the spinal fluid urea, left the hospital with little if any improvement The history of these two cases in which there was high urea content was

TABLE 3.—NEPHRITIS NOT TERMINATING IN DEATH

Case No	Age Years	Urea Pct Spinal Fluid	Diagnosis Clinical	Affecting Diagnosis	Further Examination of Spinal Fluid
1	49	0 22	Chronic uremia Chr interstitial nephritis Gen'l arteriosclerosis	Albuminuria, casts Headache, vomiting Unimproved	25 c c clear, no cells Butyric acid test negative
2	37	0 193	Chr interstitial neph Albuminuric retinitis Optic neuritis	Headache, vomiting, unconsciousness, weakness Slight albuminuria Few hyaline casts Blood Improvement slight	
3	55	0 035	Chr interstitial neph Acute uremia	Moderate edema Slight edema of retina Albuminuria marked Casts many Blood-pressure 107 208 Greatly improved	
4	24	0 025	Chr interstitial neph	History of very marked albuminuria two years previously Headache for two years Slight albuminuria Occasional casts Slight edema Greatly improved Eye-grounds normal	
5	51	0 051	Chr interstitial neph Cardiac hypertrophy	Blood pressure 180 185 Marked albuminuria Slight edema No nervous symptoms Greatly improved	
6	35	0 025	Acute nephritis Acute pharyngitis Acute bronchitis Uremia	Edema of legs and chest, nausea, dyspnea, convulsions, fundi normal, many casts Apparently cured	
7	39	0 06 0 05	Chr interstitial neph	Headache, vomiting, neuroretinitis Systolic blood-pressure 168 220 Moderate albuminuria, considerable number of casts Temporarily improved	
8	46	0 08	General diffuse neph Arteriosclerosis Staphylococcus aureus Pyemia	Death due to sepsis Necropsy	

TABLE 4.—PATHOLOGICAL CONDITIONS WITH DIAGNOSIS OTHER THAN UREMIA, FROM WHICH, HOWEVER, NEPHRITIS CANNOT BE EXCLUDED

Case No	Age Years	Urea Pct Spinal Fluid	Diagnosis Clinical	Affecting Diagnosis	Further Examination of Spinal Fluid
1	55	0 431	Cerebral hemorrhage Diabetes mellitus	Glycosuria Acidosis Urine shows heavy trace of albumin No casts Specific gravity 1 037	Blood-tinged fluid Polymorphs, 92%, lymphos, 8%
2	35	0 07	General paresis	Slight albuminuria Casts Blood, Wassermann strongly positive	Wassermann strongly positive
3	21	0 018	Nephritis (?) Neuroretinitis, both eyes Myxedema (?)	Slight albuminuria Occasional casts Blood-pressure 100 110	
4	38	0 048	Nephritis (?) Uremia (?)	Slight albuminuria Many hyaline casts No definite history	22 cc clear fluid Wassermann negative Butyric acid test negative
5	30	0 067	Dementia	Convulsions Hallucinations Blood-pressure 110 130 Slight albuminuria Casts in two of six examinations made	Polymorphs, 35%, lymphocytes, 58%, large monos, 5%, eosinophils, 2%
6	36	0 053	Old hemiplegia Broken cardiac compensation Uremia (?)	Albuminuria marked Many hyaline and granular casts Admitted moribund Irrational Temp 98 F Eye grounds normal	Polymorphs, 98%
7	2	0 358	Tuberculous *meningitis*	*Unconscious* Vomiting of two weeks' duration No examination of urine Blood-pressure not taken Survived one day in hospital	No tubercle bacilli found
8	36	0 421	Acute general tuberculosis Tuberculous meningitis	Chronic alcoholism Eyegrounds show only a blurring of discs Slight albuminuria No casts Blood-pressure 110-120 Edema none Septic temperature Irrational	No tubercle bacilli found
9	37	0 153	Atrophy of optic nerves	Headache, vomiting Unconsciousness, three attacks Slight albuminuria No casts Blood-pressure not taken	
10	8/12	0 614	Tuberculous meningitis	Duration six days Vomiting, convulsions, *coma* No Kernig Some stiffness of neck Slight dyspnea No Babinski reaction	No tubercle bacilli in three examinations of spinal fluid First lymphocytes, 74%, second lymphocytes, 42%
11	65	0 121	Chr interstitial neph ? Cardiac decompensation Senility	Trace of albumin in two of ten examinations Hyaline casts in four examinations No nervous symptoms	
12	49	0 095	Diabetes mellitus Ulcerative colitis Chronic diffuse neph General arteriosclerosis Cardiac hypertrophy	Albuminuric retinitis Blood-pressure 197-220 Marked albuminuria	

not subsequently followed, but in the light of experience the patients may be presumed to have survived only a short time

Of the twelve cases tabulated under Group 4, cases 1, 7, 8 and 10 present interesting problems Unfortunately, necropsies could not be obtained in any of them, and it is therefore impossible to exclude nephritis as a complication or indeed the principal cause of death, but we feel that the burden of proof lies with the clinician as regards diagnosis

CONCLUSIONS

Our conclusions as to the interpretation which may safely be made from increased amounts of urea in the spinal fluid must remain, both diagnostically and prognostically, almost the same as those of the authors quoted

1 A spinal fluid urea content higher than 0 2 per cent indicates a severe uremia and a rapidly fatal termination

2 A content between 0 1 per cent and 0 2 per cent means a rapidly fatal termination in the majority of cases of nephritis

3 A content between 0 05 per cent and 0 1 per cent does not permit of any definite conclusions either as regards diagnosis or prognosis Such a content is, however, suggestive of severe urea retention and must be taken into consideration in the diagnosis of the condition

4 As to diagnosis we believe that the determination of the presence or absence of urea retention in the body fluids will go far to clear up certain difficult problems where the question of uremia enters into consideration

We desire to express our thanks to the attending physicians and members of the house staff who have made this material available

41 East Seventieth Street

THE GONOCOCCUS COMPLEMENT-FIXATION TEST AND ANALYSIS OF RESULTS FROM ITS USE*

B A THOMAS, M D, AND ROBERT H IVY, M D
PHILADELPHIA

In recognition of the recent admirable work by Schwartz and McNeil on the complement-fixation test in gonococcic infections, the fact must not be overlooked that Muller and Oppenheim, in 1906, were the first to apply this reaction to a gonorrheal affection and consequently are entitled to the distinction of being termed the originators The present popularity of this test has been the outgrowth of the suggestion made by Schwartz and McNeil — namely, that of the employment of a polyvalent antigen As a result of their labors, these workers contend, and seem to have proved conclusively, through animal experimentation (1) "that the different strains of the gonococcus differ markedly one from another — so much so that the antibodies produced in the body by the toxin of one strain will in many instances not bind the complement in the presence of an antigen prepared from another strain Therefore, if only one strain is used in the preparation of the antigen, a great many negative results would be obtained in positive cases, (2) an antigen prepared from many strains fixes the complement whenever one of its component strains does so, and consequently the necessity of testing a serum against a number of antigens separately is avoided It is not to be denied that there probably are other strains of gonococci differing widely from any present in the polyvalent antigen, so that at times a negative result will be obtained in a positive case"

While we recognize the fact that a negative reaction may mean nothing, in fact, may be erroneously contradictory, the significance, on the other hand, of a positive reaction has been so great — more specific, in fact, than when the lipotropic antigen, commonly employed in the performance of the Wassermann reaction, is utilized — that we have applied the test for the past few months in a large series of diverse cases with the most gratifying results Without entering on any discussion of the theories governing fixation or deviation of complement and hemolysis, it is our purpose, at this time, merely to outline our own technic, desig-

* From the Department of Genito-Urinary Surgery of the Philadelphia Polyclinic Hospital and the William Pepper Clinical Laboratory of Medicine, University of Pennsylvania

* Read before the Philadelphia County Medical Society, May 28, 1913
* Submitted for publication Aug 8, 1913 -

nating certain points of superiority which it may possibly possess over that employed by Schwartz and McNeil, and to submit the analysis and results of cases in which serums were subjected to the gonococcus complement-fixation test

DISCUSSION OF TECHNIC

The technic utilized and advocated by Schwartz and McNeil in the performance of the complement-fixation test for gonococcic infections embodies what may be termed the minimal quantity method That is, the smallest quantities of the component substances entering into the reaction are employed that are compatible with practicability Other than as a matter of economy and questionable conservation of time, this method possesses no recommendation In fact, from a comparative study of the results in a series of cases in which Schwartz and McNeil's method is contrasted with the technic analogous to the Wassermann reaction as employed by us, it has appeared that the advantage has always rested with the latter or larger quantity method, in that the hemolytic readings seemed to be easier of interpretation [1] Moreover, we have been impressed by the fact that the quantity of antigen employed in the former case, as will be seen presently, is too great — being indeed as much as, and more than, that utilized by us with five to ten times the gross quantity of material

Schwartz's latest method and the technic which we practiced for a time in comparison with our own gonococcus complement-fixation test is as follows, so far as the quantities of the ingredients participating in the reaction are concerned (1) patients' serum, 0 02 c c , (2) salt solution, sufficient to equalize the volume in each tube, (3) antigen (routinely 0 3 c c in one tube and 0 15 c c in another tube, of a commercial preparation diluted 1 to 10) These quantities are determined by preliminary standardization with a fresh known negative and a known positive serum, the positive showing the true antigenic dose, and the negative the highest quantity of antigen which will allow complete hemolysis, in the actual test this large quantity of antigen is placed in one tube and one-half the quantity in a second tube, (4) complement 0 1 c c of a dilution 1 to 10, (5) amboceptor, 0 1 c c representing twice the lowest quantity that will completely hemolyse 0 1 c c of the cell suspension with 0 1 c c of complement in one hour, (6) sheep's red blood-cells (5 per cent suspension), 0 1 c c Incubation is made for one-half hour at 37 C

1 If it be necessary or desirable to practice economy in the performance of the test, we would suggest, in preference to the very small quantities employed by Schwartz and McNeil, which are more or less tedious to manipulate, just half of the component materials as employed in our technic We have resorted to this modification on several occasions with results just as satisfactory as those obtained by the regular method

in a water-bath or for one hour in dry heat, before and after the addition of the hemolytic system

The technic on which we have learned to place the greatest reliance is essentially that utilized by us in the performance of the Wassermann reaction, merely substituting the gonococcus specific antigen for the syphilitic lipotropic antigen employing always the carefully standardized single complement unit and the routine standardization of antigen and amboceptor Briefly, the steps in the daily procedure are as follows

AUTHORS' TECHNIC

As previously stated, a detailed description of the fundamental serological principles involved in the complement-fixation or deviation reaction, will be omitted from this discussion We shall submit, therefore, merely a table representing the routine procedure in the performance of the gonococcus complement-fixation test (Table 1)

We believe that the features of importance in the proper execution of this test are the required inactivations, the exact standardization of the hemolytic amboceptor and suspension of sheep's red blood-corpuscles, and paramountly, the complement and antigen each time the reaction is undertaken

ANTIGENS

The preparation of the various ingredients entering into the reaction will receive no consideration at this time—with one exception, namely, the antigen This, however, is of such importance with respect to the gonococcus complement-fixation test that it must receive some discussion, since on the integrity and specificity of this antigen depends the whole value of the test in gonorrheal infections The necessity of a polyvalent antigen is indisputable presumably owing to the diversity of the strains of the gonococcus The only question of importance is, how may this antigen be prepared to the best advantage? Schwartz and McNeil in their latest communication say that the "various strains of gonococci are grown on salt-free veal-agar, neutral in reaction to phenolphthalein, 24-hour old cultures are washed off the agar-slants with distilled water and the resulting suspension is heated for two hours in a water-bath at 56 C It is then centrifuged and passed through a Berkefeld filter No salt is added to this antigen until it is desired to use it, when it is made up to 0 9 per cent strength by adding one part of 9 per cent saline solution to nine parts of antigen Following Schwartz and McNeil's instructions to the letter, we have prepared monovalent, trivalent and hexavalent antigens[2] and have employed them comparatively in a large series of cases

2 In the near future we shall report the results of our studies of various gonococcal antigens, variously extracted, also the results comparatively of non-specific antigens prepared from certain Gram negative and Gram-positive diplococci, as the *Micrococcus catarrhalis*, the meningococcus, the pneumococcus, the *Streptococcus pyogenes*, etc

with the result that the hexavalent preparation gave the highest percentage of positive results and in every way appeared to be the most reliable of the three antigens Even with the hexavalent antigen,[3] prepared as described above, we have been forced, in comparative studies, to the conclusion that it has not been so useful or reliable as when prepared in the following manner Forty-eight-hour old cultures of the same six strains of gonococci, grown on blood-agar, were washed off in sterile distilled water, shaken for one hour, and autolyzed for twenty-four hours in a

TABLE 1—

	No Test Tube	Antigen (Dilution 1 10) c c	NaCl Solution (0 85%) c c	Patient's Serum (Inactivated) c c	Known Positive Serum (Inactivated) c c	Known Negative Serum (Inactivated) c c	Complement (Dilution 1 10) c c		Hemolytic Amboceptor (Antisheep) (Titre = 1 2,000) (Dilution 1 1,000) c c	Red Blood Corpuscles (Sheep's 5% Washed Suspension) c c
Tests for complement standardization	1	0 2 ?	1 3				0 2	Incubation at 37 C for one hour	1 0	1 0
	2	0 2 ?	1 2				0 3		1 0	1 0
	3	0 2 ?	1 1				0 4		1 0	1 0
	4	0 2 ?	1 0				0 5		1 0	1 0
Tests for antigen standardization and controls	5	0 05	1 5		0 1		0 4 ?		1 0	1 0
	6	0 1	1 4		0 1		0 4 ?		1 0	1 0
	7	0 2	1 3		0 1		0 4 ?		1 0	1 0
	8	0 3	1 2		0 1		0 4 ?		1 0	1 0
	9	0 2	1 3			0 1	0 4 ?		1 0	1 0
	10	0 4	1 1			0 1	0 4 ?		1 0	1 0
	11	0 6	0 9			0 1	0 4 ?		1 0	1 0
Tests and controls for the suspected serum	12	0 2	1 3	0 1			0 4		1 0	1 0
	13	0 2	0 9	0 1			0 8			
	14	0 2	1 3	0 1			0 4		1 0	1 0
	15		1 5	0 1			0 4		1 0	1 0
									1 0	1 0

thermostat at the temperature of 37 C and heated in a water-bath at 60 C for one-half hour Before use, this antigen is diluted one to ten by the addition of 0 85 per cent salt solution In spite of our efforts[2] thus far, we have been unable to produce an antigen showing results quite as clean-cut, although they have been just as constant, as that marketed by

3 We are indebted to Dr A P Hitchens of H K Mulford Co for the six strains of gonococci employed in this work It should be noted in this connection that it is assumed that the cultures are true gonococci, since their staining and morphological characteristics are identical with that micro organism, although they grow readily on blood serum or even on plain agar agar The dispute has arisen as to whether a Gram negative diplococcus growing in this wise, should or should not be designated as the *Micrococcus catarrhalis*

Parke, Davis & Co The probable explanation is that their antigen is made up from twelve strains of the gonococcus, while ours contains but six[3]

ANALYSIS OF CASES TESTED

The result of our work has been little more than a corroboration of the reports that have emanated from Schwartz and McNeil[4] and those who have confirmed their results We believe, however, that, by utilizing the technic herein described, we have added to the accuracy of the test

TEST REACTIONS

	Results (Immediately or Morning After Refrigeration)	Objects of the Reactions
Incubation at 37 C for one and one-half hours	Incomplete hemolysis	To determine quantity of complement to be used in test proper
	Incomplete hemolysis	To determine quantity of complement to be used in test proper
	Complete hemolysis	To determine quantity of complement to be used in test proper
	Complete hemolysis	To determine quantity of complement to be used in test proper
	Incomplete hemolysis	To determine quantity of antigen to be used in test proper
	Partial hemolysis	To determine quantity of antigen to be used in test proper
	No hemolysis	To determine quantity of antigen to be used in test proper
	No hemolysis	To determine quantity of antigen to be used in test proper
	Complete hemolysis	To prove that the antigenic dose is not in itself anticomplementary
	Complete hemolysis	To prove that twice the antigenic dose is not in itself anticomplementary
	Incomplete hemolysis	To prove that thrice the antigenic dose is not in itself completely anticomplementary
	No hemolysis (Positive reaction hemolysis = 1 unit)	To determine quantitatively the degree of complement fixation
	No hemolysis (Positive reaction hemolysis = 2 units)	To determine quantitatively the degree of complement fixation
	Complete hemolysis (Complete negative reaction)	Shows that there was no immune body present in the patient's serum with the aid of the antigen to fix the complement
	Complete hemolysis	Proves that the immune body itself will not fix complement

as applied by them and have thereby improved the findings to the credit of the test and its value in clinical diagnosis To the increased positive results we attribute the accurate standardization of antigen on each occasion and the employment of a standardized single complement unit For the sake of convenience, our results with respect to the serums tested, numbering in all 204, have been tabulated (Table 2)

4 Schwartz and McNeil "The Complement-Fixation Test in the Diagnosis of Gonococcic Infections," Am Jour Med Sc, May, 1911, "The Complement-Fixation Test in the Differential Diagnosis of Acute and Chronic Gonococcic Arthritis," ibid, September, 1912, "Further Experiences with the Complement-Fixation Test in the Diagnosis of Gonococcus Infections of the Genito-Urinary Tract in the Male and Female," ibid, December, 1912

TABLE 2.—RESULTS WITH RESPECT TO SERUMS TESTED

KNOWN NON GONORRHEAL AFFECTIONS

No	Remarks	Result of Gonococcus Complement Fixation Reaction — Antigens				
		Monovalent (Unfiltered)	Trivalent (Filtered)	Parke, Davis & Co (Schwartz Technic)	Hexavalent Unfiltered	Parke, Davis & Co
1	Typhoid fever				Negative	Negative
2	Typhoid fever				Negative	Negative
3	Typhoid fever				Negative	Negative
4	Tuberculous otitis media and pulmonitis				Negative	Negative
5	Tuberculous otitis media and pulmonitis					Negative
6	Pulmonary tuberculosis				Negative	Negative
7	Diabetes mellitus				Negative	Negative
8	Diabetes mellitus				Negative	Negative
9	Diabetes mellitus				Negative	Negative
10	Acute streptococcic endocarditis				Negative	Negative
11	Chorea, secondary endocarditis				Negative	Negative
12	Malaria				Negative	Negative
13	Syphilis					Negative
14	Congenital syphilis					Negative
15	Syphilis					Negative
16	Luetic rhinitis					Negative
17	Syphilis					Negative
18	Syphilis					Negative
19	Normal, no symptoms of gonorrhea				Negative	Negative
20	Normal, no symptoms of gonorrhea					Negative
21	Normal, no symptoms of gonorrhea	Negative	Negative	Negative		Negative
22	Tuberculous osteitis and pulmonitis					Negative
23	Chronic hypertrophic arthritis					Negative
24	Subacute arthritis (sacroiliac and elbow)				Negative	Negative
25	Arthritis (both ankles), six weeks				Negative	Negative
26	Tuberculous arthritis (knee)					Negative
27	Chronic synovitis (midtarsal)				Negative	Negative
28	Optic atrophy					Negative
29	Neurasthenia					Negative
30	Neurasthenia					Negative
31	Chancroid					Negative
32	Vesical calculus, cystitis					Negative
33	Hemiplegia					Negative
34	Myelitis, incontinence of urine	Negative				Negative
35	Case of rape, no signs of infection	Negative		Negative		Negative

36	Epididymitis, possibly tuberculous, but undoubtedly gonorrheal				Medium +	Medium +
37	Urine cloudy and full of shreds	Medium +				Strongly +
	PATIENTS CLINICALLY CURED					
38	Several attacks of gonorrhea, no signs at present					Weakly +
39	Convalescent after treatment for 2½ years for chronic urethritis and prostatitis					Negative
40	Syphilis, had three doses of neosalvarsan, convalescent from chronic urethritis					Negative
41	Convalescent from chronic prostatitis					Negative
42	Convalescent from chronic urethritis, three attacks, first 3 years ago					Negative
43	Convalescent from first attack of gonorrhea 8 weeks previously					Negative
44	Convalescent from attacks of gonorrhea 13 and 2 years previously				Negative	Negative
45	Gonorrhea several years ago, no symptoms now				Negative	Negative
46	Clinically convalescent from gonorrhea acquired 2 years ago					Negative
47	Convalescent from urethritis and prostatitis, received 8 c c of antigonococcic serum 15 months ago for gonorrheal pleurisy				Negative	Negative
48	Polyarthritis, has gonorrheal history					Negative
49	Gonorrhea three times, asserts a cure, complains of impotence	Medium +				Strongly +
50	Gonorrhea 3 years ago, epididymitis 9 months ago, prostatorrhea, wants to marry					Negative
51	Gonorrhea three or four times, now clinically cured					Negative
52	Gonorrheal history, apparently cured	Negative	Negative	Negative		Negative
53	Gonorrhea 5 months ago, cured					Negative
54	Contracted gonorrhea 1½ years ago, convalescent from chronic prostatitis					Negative
	CASES ACCIDENTALLY DISCOVERED CLINICALLY CURED					
55	Confessed to gonorrhea 2½ years ago under antisyphilitic treatment	Weakly +				Weakly +
56	Luetic rhinopharyngitis, no inquiry as to gonorrhea					Weakly +
	ACUTE ANTERIOR URETHRITIS					
57	Gonorrhea of 12 hours duration (first attack)					Negative
58	Gonorrhea of 10 days' duration (first attack)					Negative
59	Acute gonorrhea (first attack)					Negative
60	Gonorrhea of 11 days' duration (first attack)					Negative
61	Gonorrhea of 7 days' duration (first attack)					Negative
62	Gonorrhea of 4 weeks' duration (first attack)				Negative	Negative

TABLE 2.—RESULTS WITH RESPECT TO SERUMS TESTED.—(Continued)

No	Remarks	Result of Gonococcus Complement-Fixation Reaction — Antigens				
		Monovalent (Unfiltered)	Trivalent (Filtered)	Parke, Davis & Co (Schwartz Technic)	Hexavalent Unfiltered	Parke, Davis & Co
	ACUTE AND SUBACUTE ANTERO POSTERIOR URETHRITIS					
63	Gonorrhea of 6 weeks' duration (first attack)					Medium +
64	Gonorrhea of 5 weeks' duration (first attack)					Negative
65	Gonorrhea of 4 weeks' duration (first attack 10 years ago)					Negative
66	Gonorrhea of 2 weeks' duration (first attack)					Negative
67	Gonorrhea of 10 days' duration (first attack 1 year ago					Negative
68	Gonorrhea of 7 weeks' duration (first attack)					Negative
69	Gonorrhea of 4 weeks' duration (first attack)				Negative	Negative
70	Gonorrhea of 6 weeks' duration (first attack)				Negative	Negative
71	Gonorrhea of 7 days' duration (three previous attacks)				Negative	Negative
72	Gonorrhea of 10 days' duration (six previous attacks)				Negative	Negative
73	Gonorrhea of 5 week's duration (first attack 2 years ago)					Negative
	CHRONIC URETHRITIS PLUS ACUTE EXACERBATION					
74	Gonorrhea 1 year previously, received 8 c c of antigonococcic serum, duration of present attack is 4 weeks					Weakly +
75	Gonorrhea 2 years previously, duration of present attack is 4 weeks					Medium +
76	Gonorrhea 1 year previously, duration of present attack is 3 weeks					Strongly +
77	Gonorrhea 1 year ago, acute exacerbation at present					Weakly +
78	Gonorrhea 8 times previously, duration of present attack is 17 days	Strongly +	Weakly +	Strongly +		Strongly +
79	Gonorrhea 5 years ago, duration of present attack 10 days				Weakly +	Weakly +

80	Gonorrhea 4 years ago present attack of 8 months' duration					Medium +
81	Gonorrhea several times, present attack of 2 months' duration					Negative
82	First attack, duration of 6 months					Negative
83	Contracted gonorrhea 3 years ago					Weakly +
84	First attack, duration 3½ months					Negative
85	First attack duration 6 months					Negative
86	First attack duration 2½ months					Strongly +
87	Gonorrhea twice previously present attack 3 months duration					Weakly +
88	First attack duration 8 months					Negative
89	Gonorrhea several times urine contains shreds					Negative
90	First attack duration 5 months					Negative
91	Chronic gonorrhea duration unknown					Negative
92	Gonorrhea 7 times in 12 years, duration of present attack unknown					Strongly +
93	Gonorrhea 4 years ago, duration of present attack unknown					Medium +
94	Gonorrhea 2 times previously, duration of present attack 2 years	Medium +		Medium +		Medium +
95	Gonorrhea 1½ years ago, present attack 5 months duration					Negative
96	Gonorrhea 16 years ago, present attack of 9 months' duration					Negative
97	First attack, duration 1 year					Negative
98	First attack duration 4 months					Negative
99	Urethritis for 12 years					Negative
100	Chronic urethritis, duration unknown					Strongly +
101	Gonorrhea one year ago pain in heel					Negative
102	Chronic recurrent urethritis	Negative	Negative	Negative		Negative
103	Gonorrhea 2 years ago, duration of present attack unknown					Negative
104	Gonorrhea 2 years ago slight morning discharge					Negative
105	Gonorrhea 3 times previously present attack 3 months duration					Weakly +
106	Gonorrhea 5 years ago, present attack 4½ months' duration					Strongly +
107	Gonorrhea previously duration of present attack 6 months				Weakly +	Weakly +
108	Gonorrhea 3 years ago, bilateral buboes					Negative
109	First attack duration 15 months, had four inoculations of Gonorrhea Bacterin 1 year ago				Negative	Negative
110	Chronic urethritis duration unknown				Negative	Negative
111	Gonorrhea for years several recurrences				Negative	Negative
112	First attack duration 2 months				Negative	Negative

TABLE 2—Results with Respect to Sirums Tested—(Continued)

No	Remarks	Result of Gonococcus Complement Fixation Reaction — Antigens				
		Monovalent (Unfiltered)	Trivalent (Filtered)	Parke, Davis & Co (Schwartz Technic)	Hexavalent Unfiltered	Parke, Davis & Co
113	Recurrent, duration at least 1 year					Weakly +
114	Second attack gonorrhea 1 year ago, prostatitis and seminal vesiculitis treated for 1 year, practically cured at present					Negative
115	Duration 6 months					Negative
116	Gonorrhea 4 years ago, recurrent prostatitis, seminal vesiculitis and stricture ever since					Weakly +
117	Gonorrhea 1 year ago and 5 months ago				Medium +	Medium +
118	First attack of gonorrhea 1 year ago, never cured	Negative				Strongly +
119	Gonorrhea 8 years ago, not treated, now has shreds in urine					Negative
120	Gonorrhea 6 years ago, discharge at present					Strongly +
121	First attack of gonorrhea 1 year ago, chronic posterior urethritis and prostatitis					Medium +
122	First attack of gonorrhea 3 months ago					Negative
123	Duration uncertain					Weakly +
124	Has had gonorrhea nine times, last attack 6 months ago	Negative	Negative	Negative		Negative
125	First attack of gonorrhea 9 years ago, last attack 2 years ago					Negative
126	Duration unknown					Negative
127	Gonorrhea 3 years ago and continuously ever since					Medium +
128	Gonorrhea past 4 years, second attack					Negative
129	First attack of gonorrhea 5 years ago, never cured					Doubtful
130	Duration unknown	Medium +				Strongly +
131	Gonorrhea twice before, present attack of 4 years' duration	Medium +		Medium +		Strongly +
132	Gonorrhea 9 months ago, discharged as cured from mother hospital, still has shreds in urine					Weakly +
133	Duration one year					Negative
134	Gonorrhea five times					Medium +
135	Duration unknown					Negative
136	Gonorrhea four times, last time 8 months previously					Weakly +
137	Gonorrhea 4 years ago, last attack 2 months ago					Negative

138	Ten or twelve attacks of gonorrhea, last attack 6 months ago					Negative
139	Gonorrhea 2 years previously, shreds in urine					Weakly +
140	Gonorrhea one year ago	Weakly +	Weakly +			Strongly +
141	First attack, duration 1 year					Negative
142	Chronic hypertrophic changes in knee joint, prostatitis	Negative				Negative
143	Gonorrhea for four years					Strongly +
144	Gonorrhea contracted 5 years ago, now has mild prostatitis and stricture					Negative
145	Gonorrhea 4 years ago, now has prostatitis and seminal vesiculitis				Negative	Negative
146	First attack of gonorrhea 6 years ago, present recurrence one year ago (mild)					Negative
147	Duration unknown, has taken gonorrheal phylacogen without benefit					Medium +
148	Several attacks of gonorrhea over period of 10 years, had forty-four inoculations of gonococcic bacterin in 6 months 2 years ago					Weakly +
149	Same case 1 week later, under treatment					Negative
150	Gonorrhea 2 years ago					Negative
151	Gonorrhea first time 5 years ago, twice since, duration of prostatitis 1½ years, three recurrences, last one 9 months ago					Negative
152	Recurrence of above case 2 months later					Negative
153	Gonorrhea 26 months ago, very slight, involvement of prostate, urine clear				Negative	Negative
154	Duration unknown, case 155, 10 days after treatment	Negative		Negative		Negative
155	Gonorrhea 5 years ago					Weakly +
156	First attack, duration 6 months					Weakly +
157	Above case three weeks later					Weakly +
158	Gonorrhea 3 years previously					Weakly +
159	Gonorrhea 15 years ago, last 2½ years ago, premature ejaculation	Weakly +	Weakly +			Strongly +
160	Gonorrhea 7 and 2 years, present attack of 4 mos' duration, had 5 injections of phylacogen					Medium +
	STRICTURE					
161	Gonorrhea 3 years and 4 months ago					Medium +
162	Gonorrhea 14 and 12 years ago	Negative	Negative	Negative		Negative
163	Gonorrhea for 1½ years					Negative
164	Two attacks of gonorrhea, last one 18 months ago	Negative		Strongly +		Weakly +
165	Gonorrhea for 4 years with recurrences				Strongly +	Medium +
166	Duration unknown					Strongly +

TABLE 2.—RESULTS WITH RESPECT TO SERUMS TESTED.—(Continued)

No	Remarks	Result of Gonococcus Complement-Fixation Reaction — Antigens				
		Monovalent (Unfiltered)	Trivalent (Filtered)	Parke, Davis & Co (Schwartz Technic)	Hexavalent Unfiltered	Parke, Davis & Co
		EPIDIDYMITIS				
167	Duration three weeks					Negative
168	Same as 167, two weeks later					Weakly +
169	Epididymitis 2½ months ago, still unresolved, prostatitis					Medium +
170	First attack of gonorrhea, duration unknown					Medium +
171	Gonorrhea and epididymitis 2 years ago, second attack at present	Weakly +	Weakly +			Strongly +
172	Gonorrheal history, slight swelling of epididymis	Negative	Negative			Medium +
173	Previous attack of gonorrhea, present attack of six weeks' duration					Medium +
174	Gonorrhea three or four times, last time six weeks ago					Weakly +
175	First attack of gonorrhea, duration six months	Negative	Weakly +	Strongly +		Strongly +
176	Gonorrhea twice previously, present attack of six weeks' duration	Negative	Strongly +			Strongly +
177	Acute attack of nine days' duration					Weakly +
178	Acute attack of seven days' duration, denies gonorrhea					Medium +
179	Gonorrhea three times previously, epididymitis 15 years ago, plus present attack					Strongly +
180	Duration two months	Strongly +	Medium +			Strongly +
181	Duration unknown					Medium +
182	Bilateral epididymitis, probably tuberculous, has pulmonary tuberculosis, gonorrhea 10, 5 and 2 years ago					Negative

183	Chronic hypertrophic arthritis, duration 1 year, abdominal section 3 years ago, no G U symptoms at present					Medium +
184	Arthritis knee for 10 years, urethritis for 6 months, since when knee has been worse					Weakly +
185	Multiple subacute arthritis, no history of gonorrhea	Weakly +		Weakly +		Weakly +
186	Arthritis both ankles, gonorrhea for 3 years, synovitis 3 weeks after gonorrhea					Weakly +
187	Duration 10 weeks				Weakly +	Weakly +
188	Pain in left shoulder, no inquiry as to gonorrheal history					Weakly +
	GYNECOLOGICAL CASES					
189	Pain in right ovarian region, leukorrhea, dysuria, frequency					Negative
190	Eclampsia				Negative	Negative
191	Puerperal sepsis				Negative	Negative
192	Pelvic abscess, puerperal sepsis				Weakly +	Weakly +
193	Abscess of uterine cornua, vaginal smear shows Gram diplococci				Negative	Negative
194	Abscess in cornua of uterus, delivered of dead fetus				Negative	Negative
195	Leukorrhea, dysuria, frequency				Negative	Negative
196	Vaginal smear shows Gram diplococci				Negative	Negative
197	Vaginal smear shows Gram-diplococci				Negative	Negative
198	Tenderness in hypogastrium, vaginal smear shows Gram-diplococci				Negative	Negative
199	Tenderness in hypogastrium, vaginal smear shows Gram diplococci				Negative	Negative
200	Salpingitis					Negative
201	Pyosalpingitis				Negative	Negative
202	Gonorrheal urethritis, salpingo oophorectomies					Medium +
203	Bartholinitis and cyst	Negative				Negative
204	Gonorrheal endometritis					Strongly +

Reviewing our experience with the gonococcus complement-fixation test in general, it may be stated that a negative reaction is not decisive against the presence of a gonorrheal infection, and this is particularly true during the first six weeks of a primary acute urethritis either anterior or posterior in the absence of any complication, previous to which time we have never obtained a positive reaction, on the other hand, the supervention, even during the acute stage of the disease, of complications such as epididymitis arthritis, prostatitis, etc, is prone to result in the production of a positive reaction On the contrary in our experience, a positive reaction has been pathognomonic of a focus of gonococcal infection and has assisted many times in elucidating obscure or doubtful lesions In fact, it appears that the gonococcus-fixation test enjoys greater specificity than does the Wassermann reaction, since thus far we have found no alien infection or condition capable of producing a positive reaction This much certainly cannot be claimed for the Wassermann reaction Moreover, there is no drug, as there is in syphilis, which is capable of causing the reaction to be negative during the existence of the disease The probable explanation for the greater specificity of the gonococcus complement-fixation test rests in the fact that with gonococcic infections we employ a specific antigen—the gonococcus—while in the case of syphilis a non-specific or lipotropic antigen is employed

The analysis of our cases further illustrates another interesting feature, namely, the persistence in some cases for a short time of a positive reaction, after an apparent clinical cure This has occurred so often that we no longer discharge a patient cured or give him a clean bill of health so long as he gives a positive reaction, provided he has not been the recipient of immunotherapy Usually a persistent positive reaction will become negative in two or three weeks following clinical cure with or without a continuation of treatment The only explanation is that it requires an indefinite time for the antibodies, formed during the course of infection, to disappear from the blood Torrey,[5] in animal experimentation, has found that the antibodies in immunized rabbits begin to disappear after ten days, and that the elimination is practically complete by the fiftieth day in all cases, disappearing much earlier in many instances Thus a patient, evidencing a positive reaction two months after presumed clinical cure, should be regarded as still harboring a latent gonorrheal focus Such experience, adopted either as routine procedure in the management of treatment, or discovered accidentally when gonorrheal infection or its symptoms were denied, or demonstrated by submitting suspected or positive syphilitic serum to the gonococcus-fixation test, has been encountered in a large number of cases

Because of the generally acknowledged difficulties, at times, of differentiating the pelvic lesions in women, notably certain of the inflammatory

5 Torrey Jour Med Research, 1910, No 1

from the cystic and neoplastic conditions, and also the differential diagnosis among gonorrheal, tuberculous and pyogenic infections themselves, the gonorrheal-fixation test seems destined to play a rôle As in the male, in whom a positive reaction seems never to occur, at least so long as the infection is confined to the anterior urethra, so in the female we have been unable to obtain a positive reaction unless the disease has ascended to the level of the uterus

An interesting, if not important, feature connected with this work is the comparative importance and value of the serological and bacteriological examination of cases of suspected gonorrheal infection It is, to-day, a fact that the judiciary courts of our land require that the presence of the gonococcus be demonstrated, culturally, in order to establish its indisputable and legal identity Based on this qualification, there are many cases of gonococcic infection impossible to determine, and we do not hesitate to state that, in our judgment, many such cultures are in reality the *Micrococcus catarrhalis* and not the gonococcus This applies particularly to such isolation of the diplococcus of Neisser from chronic inflammatory processes Moreover, it must be generally recognized that the demonstration of a Gram-negative diplococcus in smear is often insufficient and faulty evidence on which to base a diagnosis of gonococci Therefore, it is most fortunate that in the chronic stage of the disease with complications, the complement-fixation test seems to be signally meritorious, while in the acute, subacute and frequently in the chronic forms of the diseases when the gonococcus may be demonstrated bacteriologically, the serological test promises little or nothing

CONCLUSIONS

Detailed and careful analysis of the gonococcus complement-fixation test, performed with the serums of the cases tabulated in our series, would seem to justify the following assertions

1 A positive reaction is invariably reliable and always denotes the presence of a focus of gonococcic infection

2 A negative reaction frequently fails to determine the presence of disease especially in the acute and subacute stage when the disease is limited to the urethra, and never when it is confined to the anterior urethra or vagina alone

3 In no alien non-gonorrheal infections of systemic disease has a positive reaction been obtained, the test, therefore, appears to be absolutely specific

4 A positive reaction has been found to be present in 21 05 per cent of patients clinically cured Such patients, therefore, should not be discharged from treatment or observation until a negative reaction has been obtained

5 Not infrequently, either when suspicious lesions are presented or accidentally, positive reactions will be discovered in patients denying gonorrhea

6 In only 9 09 per cent of cases of acute and subacute anteroposterior urethritis has the complement-fixation test resulted positively The earliest appearance of a positive reaction in a primary attack of posterior urethritis, without complication, occured in the sixth week

7 In a number of cases of chronic recurrent urethritis with acute exacerbations, the test was invariably positive, many of these patients undoubtedly had prostatitis

8 The reaction resulted positively in one-third of all cases of chronic posterior urethritis, undoubtedly many of these cases had a mild or low-grade prostatitis

9 In 52 08 per cent of cases of chronic prostatitis a positive reaction was obtainable

10 Two-thirds of all stricture cases demonstrated a positive test

11 In epididymitis a positive complement-fixation test was observed in 87 5 per cent of cases If, from our series, one case probably tuberculous, may be eliminated, and a time duration of five weeks can be imposed, the positive result in this form of disease has been 100 per cent

12 In arthritis, undoubtedly gonorrheal in character, positive reactions were obtained in 100 per cent of cases

13 In the diagnosis and differential diagnosis of pelvic disease in women, the gonococcus-fixation test is destined, unquestionably, to play an important rôle We have been unable to obtain any positive results in uncomplicated urethritis, vulvovaginitis and Bartholinitis, and it would appear that the infection must ascend at least to the level of the uterus in order to produce a positive blood response

14 Inoculations of gonococcus bacterin, antigonococcic serum, etc, may in themselves by the production of immune bodies be causes of positive reactions How long these immunizing effects may endure is unknown, but we have observed patients, treated by immunotherapy, who one year later demonstrated negative complement-fixation reactions

15 Although the bacteriological demonstration of the gonococcus culturally is the only absolute method for its identification in chronic inflammatory processes, the method as a routine procedure is impractical and susceptible of many failures and fallacious results, so that the complement-fixation test is not only less laborious, but is productive of a higher percentage of positive findings

16 Finally, we hope and trust that the complement-fixation tests in gonococcic infections, as the Wassermann reaction in syphilis, are demonstrating their reliability and value to the extent that they will be recognized as indispensable, so soon as the courts shall rule that each applicant for marriage licensure must produce a health certificate properly attested

A COMPARISON OF THE EXCRETORY POWER OF THE SKIN WITH THAT OF THE KIDNEY THROUGH A STUDY OF HUMAN SWEAT *

H W PLAGGEMEYER, M D, AND E K MARSHALL, JR, PH D
BALTIMORE

The fact that the skin and the kidneys can act, to a certain extent, vicariously, seems to be generally accepted, as is manifest by the use of sweat-baths in the treatment of uremia The skin excretes, qualitatively, practically the same substances which occur in the urine, namely, urea, ammonia, uric acid, amino-acids,[1] creatinin,[2] chlorids, phosphates, sulphates and certain enzymes [3] The quantity of these substances, however, excreted by the skin is, normally, far less than that eliminated through the kidneys

In health the ratio of the quantities of the various urinary constituents to one another is fairly constant, independent of the actual amounts of the substances eliminated by the kidney Moreover, this ratio for urine differs from that existing between the same substances in the blood-stream Hence the kidney exercises a selective concentrating action for certain substances The question then suggests itself as to whether or not any such relation exists between the normal constituents of sweat, and, further, if any definite relation obtains between the quantities of the substances eliminated through the skin and kidneys Such a similar relationship of the urinary and sweat factors would tend to show a similar selective power between the skin and kidneys

Favre and later Kast[4] studied the relationship between the chlorids, phosphates and sulphates in the urine and sweat The results show no outspoken relationship between the mineral constituents of the two fluids These investigators found the ratios shown in Table 1 between these substances in sweat and urine

Aliprando Moriggia[5] has noted the similarity of substances excreted both in sweat and urine, but showed the presence of an acid sweat in experimental alkaluria in man, while in herbivora an experimental

* From the Genito-Urinary Clinic of the Johns Hopkins Hospital and the Laboratory of Physiological Chemistry of the Johns Hopkins University
* Submitted for publication, Aug 7, 1913

1 Embden and Tachau Biochem Ztschr, 1910, xxviii, 230
2 Capranica S Maly's Jahresb, 1882, xii, 190
3 Gaube, M J Compt rend Soc de Biol, 1891, xliii (Addenda), 115
4 Kast Ztschr f physiol Chem, 1887, xi, 501
5 Moriggia A Moleschott's Untersuchungen zur Naturlehre, 1876, xi, 129

acid urine was always accompanied by alkaline sweat This would tend to show some distinct selective power of the skin and kidneys, but is not conclusive, as the reaction of sweat may depend on the admixture of secretion from the sebaceous glands [6]

TABLE 1.—CHLORIDS, PHOSPHATES AND SULPHATES IN URINE AND SWEAT ACCORDING TO STUDIES OF FAVRE AND KAST

	Chlorids	Phosphates	Sulphates	
Favre	1	trace	0 043	sweat
	1	0 0959	0 380	urine
Kast	1	0 0015	0 009	sweat
	1	0 1320	0 397	urine

W Mironowitsch[7] found more mercury excreted in the sweat than in the urine after subcutaneous injection of mercury salt during treatment for syphilis Argutinsky,[8] in an examination of two samples of sweat obtained by means of a vapor bath, determined the urea nitrogen, ammonia nitrogen and total nitrogen The urea nitrogen amounted to 68 4 per cent and 75 0 per cent of the total nitrogen, and the ammonia nitrogen to 3 1 per cent and 5 8 per cent of the total W Camerer, Jr,[6] in two cases found the urea nitrogen to be 34 6 per cent and 34 0 per cent, and the ammonia nitrogen 8 0 per cent and 6 6 per cent of the total [1]

J H Hoelscher[9] undertook a rather comprehensive study of the chemical composition of human sweat His method of obtaining sweat consisted in wrapping the subject in sterile gauze, exposing to a dry heat of not more than 120 F for a period of one or two hours, and then subjecting the gauze to pressure to obtain the fluid The specific gravity, reaction toward litmus, total solids, organic and inorganic, urea and total nitrogen are reported, but the fact that the methods adopted for the analyses are nowhere mentioned considerably lessens the value of this work Calculations which we have made from his data on the relationship between the urea nitrogen and total nitrogen of the sweat in twenty-four cases of normal passive sweat show the urea nitrogen to vary between 36 per cent and 100 per cent of the total The majority of the values show a more constant relationship between urea and total nitrogen, but the extreme variation before noted is untenable Moreover, his figures

6 Trumpy, D and Luchsinger, B Arch f d ges Physiol (Pflüger's), 1878, xviii, 494 Arloing, S Lyon méd, 1896, No 50 Camerer, Jr, W Ztschr f Biol, 1901, p 1271

7 Mironowitsch W Medic Koje Obostenze, No 12 (through St Petersburg med Wchnschr Beilage, 1895, 39)

8 Argutinsky Arch f d ges Physiol (Pflüger's), 1890, xlvi, 594

9 Hoelscher, J H Jour Am Med Assn, 1899, xxxii, 1352, N Y Med Jour, 1904, lxxix, 296

are higher in general than those which were obtained later by L Riggs[10] and also by us

Recently Riggs has made a short study of six normal cases and three nephritics[11] on the partition of the nitrogen of the sweat The total nitrogen, urea and ammonia were determined, as well as certain inorganic constituents We cite here the relationship existing between the ammonia nitrogen and urea nitrogen, as percentages of the total nitrogen, as presented in his report

TABLE 2—PERCENTAGES OF THE TOTAL NITROGEN[12] AS CALCULATED BY RIGGS

Urea Nitrogen	Ammonia Nitrogen
47 3	8 1
63 6	9 1
52 0	14 0
54 8	5 5
57 7	7 0
48 0	7 2

Tichborne claims to have detected uric acid in sweat obtained in a Turkish bath, using the murexid test[13] Hoelscher states that uric acid is not a constituent of normal sweat Riggs tested 400 c c of sweat according to Salkowski's method, but found no indication of uric acid or purin bodies Uric acid appears to have been recognized as a constituent of pathological sweats, however, that is, in gout and rheumatism[14]

In the course of this investigation, eight cases of normal passive sweat have been examined for uric acid and its presence demonstrated in each instance

Our experiments have embraced a determination of the nitrogenous constituents only, with the addition of an estimation of the diastatic activity The age and weight of the subject, the quantity of sweat eliminated in twenty-five minutes and the increase in body temperature during the procedure were noted in order to ascertain *if any relation* exists between these factors The total nitrogen, urea nitrogen and ammonia nitrogen, with the addition of uric acid and diastase, in some specimens, were estimated The normal values and relationship between

10 Riggs, Louis Jour Med Research, 1911, xix, 285

11 Although twenty six analyses are reported on nephritic sweat, these specimens of sweat were in reality obtained from three patients who were subjected to repeated sweat baths

12 This author has calculated the combined urea and ammonia nitrogen as percentages of the total, and has laid no emphasis on the percentages of the relative values here given

13 Tichborne, C R C Brit Med Jour, 1887, ii, 1097

14 Hammersten Mandel Text-Book of Physiological Chemistry, Ed 6, p 800

TABLE 3.—CHEMICAL DETERMINATIONS IN SWEAT BEGAN WITHIN AN HOUR AFTER OBTAINING THE SAMPLE

Case No	Age	Weight	Appearance time	Duration minutes	Temperature before	Temperature after	Amount obtained c c	Specific Gravity	Reaction	Total N per cent	Urea N Per Cent	Ammonia N Per Cent	Uric Acid Per Cent	Diastase $D^{38}/_{24}$
1	23	220	30	25			330	1 009	Acid	0 194	0 132	0 0159		
2	28	180	25	45			102	1 009	Acid	0 136	0 077	0 0098		
3	22	165	20	25	98 4	99 8	50	1,007	Acid	0 067	0 033	0 0084		
4	20	185	20	25	98 0	99 6	93	1 011	Neutral	0 107	0 056	0 0056		
5	23	220	15	25	98 4	99 8	323	1 008	Acid	0 085	0 034	0 0073	0 00012	
6	25	125	25	25	98 4	102 6	93	1 015	Acid	0 118		0 0087		
7	20	175	20	25	98 4	100 6	190	1 009	Neutral	0 070	0 035	0 0053		
8	26	170	20	25	98 4	100 6	175	1 007	Acid	0 068	0 035	0 0070	0 00018	
9	21	185	20	25	98 6	99 8	64	1 007	Acid	0 105	0 059	0 0094		
10	37	160	25	25	98 0	98 2	260	1 009	Neutral	0 069	0 039	0 0059	0 00014	
11	22	178	30	25	98 4	101 0	120	1 009	Acid	0 175	0 102	0 0104		
12	24	175	20	25	98 2	100 2	420	1 007	Acid	0 059	0 031	0 0056	0 00009	
13	25	190	20	25	98 2	98 8	196	1 008	Acid	0 090	0 041	0 0053	0 00014	1 1
14	34	190	15	25	98 5	99 8	270	1 007	Acid	0 055	0 026	0 0047	0 00010	1 0
15	22	185	15	25			245	1 011	Neutral				0 00005	2 1
16	23	165	20	25			340	1 007	Neutral				0 00007	1 0
17	28	210	20	25	98 2	98 8	80	1 009	Neutral					1 1
18*	22	190	20	27			180	1 008	Acid	0 127	0 078	0 0115		0 6

* During the procedure of sweating this case, 5 gm of urea, dissolved in 100 c c of distilled water were injected by gravity intravenously, during a period of three minutes The blood and urine of the subject were collected immediately before and shortly after the sweating No appreciable increase of the amount of urea present was found in either fluid after the injection

these substances in blood and in urine are well recognized Consequently, a comparison of the relationships with those of the sweat should prove instructive

EXPERIMENTAL METHODS

Method—The subjects chosen were normal male adults, living on a mixed diet, not rich in proteins The cardiorenal and gastro intestinal tracts were within range of normal, and none of the subjects were of neurotic type The experiments were performed at approximately the same hour, about four hours after a breakfast, consisting of eggs, bread and coffee, no abnormal exercise having been indulged in in the interim After rubbing the skin with 95 per cent alcohol, the subject was placed in a rubber bag 7 feet long by 4 feet in circumference, on a simple wicker couch, the upper end of which was raised 8 inches The bag itself was then caught up where necessary by large clamps to conform closely to the body of the subject, thus minimizing the air-space around him, and reducing the possible error of evaporation to a minimum Above this bag, and surrounding it entirely, was a rubber-lined air-chamber, 1 by 3 by 6 feet, into the side and top of which was projected the mouth of a pipe 6 inches in diameter, conducting hot air The end of the hot-air tube was protected below by a fan-shaped cuff of cardboard to prevent the possibility of heat being unduly concentrated No water was given the subject immediately before sweating Temperatures were taken by mouth at the beginning and end of the experiment From the time of appearance of perceptible sweat, to the conclusion of the procedure an arbitrary period of twenty-five minutes was chosen, and in most cases this was amply sufficient to secure an amount of fluid large enough for all chemical investigation

In every case the sweat was filtered through ordinary filter-paper to remove any cast-off epidermal débris As used for investigation, it was a slightly opalescent liquid Examination of several specimens microscopically gave no evidence whatever of cellular content The chemical determinations were undertaken within an hour after obtaining the sample

Methods of Analysis—Specific gravity was estimated by hydrometer Each sample was tested as to its reaction toward litmus paper The total nitrogen was determined in the usual way by the Kjeldahl-Gunning method, the urea by the method recently developed by one of us for blood,[15] and the ammonia by the aeration method[16] The uric acid determinations were made by the colorimetric method recently described by Folin for the estimation of uric acid in the blood,[17] using 100 c c to 200 c c for each determination The diastase determinations were made by the method of Wohlgemuth[18] The principle of this method depends on estimating the smallest quantity of the fluid to be tested which can completely change 5 c c of 1 per cent starch solution (Kohlbaum's *Starke loslich*) in twenty-four hours at 38 C, to products not capable of giving any blue color with iodin In the case of sweat, owing to the small amount of diastase present, we have used a 0 5 per cent or 0 25 per cent starch solution, and referred the value to a 1 per cent solution That this procedure gives reliable results was determined by duplicate experiments, using larger quantities of sweat and a 1 per cent starch solution The diastatic activity of the urine and blood-serum was estimated by the same method The values for diastase are expressed in terms of the number of cubic centimeters of a 1 per cent starch solution which 1 c c of the fluid in question can change in twenty-four hours at 38 C to products not capable of producing any blue color with iodin This is designated as $D^{38}/_{24}$

15 Marshall, Jr, E K Jour of Biol Chem, 1913, xv, 487
16 Folin Ztschr f Physiol Chem, 1902-03, xxxvii, 2261
17 Folin Jour Biol Chem, 1913, xiii, 469
18 Wohlgemuth Biochem Ztschr, 1908, ix, 1

RESULTS

Table 3 gives a general summary of the results obtained A uniformity of the time necessary to produce perceptible sweat is evident The figures cited would suggest that body-weight does not always effect a proportionate change in the amount of sweat or total nitrogen eliminated Case 6 shows an amount of 93 c c and 0 1097 gm of nitrogen eliminated for a body-weight of 125 pounds, while Case 3 shows an amount of only 50 c c and 0 0385 gm of nitrogen eliminated for a body-weight of 165 pounds No definite relationship apparently exists between the amount of nitrogen eliminated and an increase in body temperature during the procedure Case 5 shows a very high output of nitrogen (0 2758 gm) and no marked rise of temperature (99 8 F), while Case 6 shows a comparatively lower output of nitrogen (0 1097 gm) and a temperature of 102 6 F Though this test was not carried out on the same subject, we feel that the observation is of value, as it is corroborated so many times throughout the table

Although the absolute amount of any one constituent, normally eliminated, may vary between rather wide limits in different specimens there exists a fairly constant ratio between the amount of urea, ammonia and total nitrogen excreted

From the numerous investigations by Boland, Schultze, Camerer, Voges, Morner-Sjoqvist, Gumlich, Bodtker, Folin[19] and others on the relationship of different nitrogenous constituents to each other in normal *urine,* it is shown that the urea nitrogen amounts to from 84 to 91 per cent and the ammonia nitrogen from 2 to 5 per cent of the total nitrogen The relation between the total nitrogen, urea nitrogen and ammonia nitrogen of *sweat* calculated from figures in Table 3 is shown in Table 4

The urea nitrogen varies from 45 6 to 68 per cent of the total, with a mean of 53 7 per cent , while the ammonia nitrogen amounts to from 5 2 per cent to 12 4 per cent of the total, with a mean of 8 1 per cent Hence it is apparent that the relationships between the different forms of nitrogen in sweat and urine are entirely different In regard to the relationships existing between the various forms of non-protein nitrogen in *blood,* satisfactory data are far too scanty to draw any general conclusions The urea nitrogen in normal blood amounts roughly to about 50 per cent of the total non-protein nitrogen, while no apparent relationship between the amount of uric acid and urea or total non-protein nitrogen seems to exist[20] The figures for uric acid show a very different relationship in sweat from that in blood and in urine, although the data are

19 Hammersten-Mandel Text-Book of Physiologic Chemistry, Ed 6, p 646 for a collection of the literature

20 Folin Jour Biol Chem , 1913, xiv, 33

insufficient to tell whether or not any constant relationship exists between the uric acid and the total nitrogen of sweat

The concentration of urea in blood is normally about 0 02 or 0 03 per cent The kidney has the power of concentrating this about one hundred times, as the normal concentration in the urine is about 2 per cent The sweat-glands can also concentrate urea, though to a much less degree, the concentration in sweat being about 05 to 0 3 per cent. Ammonia exists in extremely minute amounts normally in the blood, and in much greater concentration both in sweat and in urine, so both skin and kidney can concentrate ammonia Uric acid exists normally in the blood in quantities ranging between 0 0007 and 0 0037 gm per 100 c c ,[21] in urine[22] between 0 015 and 0 080 gm per 100 c c , while in

TABLE 4 —PERCENTAGE OF TOTAL NITROGEN FOUND IN THE FORMS OF UREA NITROGEN AND AMMONIA NITROGEN, RESPECTIVELY

Case No	Urea nitrogen	Ammonia nitrogen
1	68 0	8 2
2	56 6	7 2
3	49 7	12 4
4	52 6	5 2
5	46 6	8 5
6		7 4
7	49 9	7 5
8	51 5	10 2
9	56 0	8 9
10	55 9	8 4
11	58 3	5 9
12	52 2	9 5
13	45 6	5 8
14	47 3	8 5
18	61 4	9 0

sweat we have found only from 0 00005 to 0 00018 gm per 100 c c From this it is evident that the kidney does normally concentrate uric acid, while in the passage of fluid through the skin its concentration is much reduced The concentration in sweat is only about one-twentieth that in the blood

Diastase, in general, may be said to occur in the urine in greater concentration than in the blood,[23] while the figures for sweat show a diastatic activity much lower than that of either blood or urine Owing to the wide variation of diastatic activity of the blood, it was thought interesting to confirm this point by making diastase determinations on the blood, urine and sweat of the same subjects The results show that,

21 Folin and Denis Jour Biol Chem , 1913, xiv, 29
22 Folin and Denis Jour Biol Chem , 1912, xiii, 363
23 Wohlgemuth Biochem Ztschr , 109, xxi, 381, 432

although the kidney normally concentrates the enzyme, the latter is much less concentrated in the sweat than in the blood

The urine was collected immediately before and after sweating, and the blood withdrawn from the arm immediately after emergence from the bag The values show the amount in cubic centimeters of 1 per cent starch solution, which 1 c c of the fluid can change to products not capable of giving blue color with iodin in twenty-four hours at 38 degrees

TABLE 5 —DIASTATIC DETERMINATION ($D^{38}/_{24}$) IN URINE, BLOOD AND SWEAT

	Urine		Blood	Sweat
Case No	Before	After		
13	1,000	167	167	1 1
14	100	100	25	1 0
17		166	25	1 1

COMPARISON OF THE EXCRETION OF DYES THROUGH THE SKIN AND KIDNEY

As far as concerns the excretion of non-toxic dye-stuffs through the skin, the following data show that the skin does not normally excrete the faintest trace of the substances used, which substances pass readily into the urine Phenolsulphonephthalein was administered intramuscularly in 6 mg doses in four normal individuals, the injection was made immediately before entering the bath, and the drug began to appear in the urine before the appearance of perceptible sweat and continued to be excreted after the sweating process was completed In each of these cases the output of phthalein was normal in the urine, but not the slightest trace could be detected in the sweat even after concentration [24] Experiments with methylene-blue were conducted in two ways In one case, 8 grains were given in 4-grain doses, one hour apart, the last dose being given within thirty minutes of the time of appearance of perceptible sweat Although the urine was deeply colored, both before and after collecting the sweat, no trace of methylene-blue could be found in the latter In two other instances in which the subject had been given 12 grains of the drug per day for three days preceding the experiment, no

24 In a series of forty cases of universal skin lesions of all types, including dermatitis exfoliativa, psoriasis universalis, eczema, ichthyosis, etc, studied by one of us (Plaggemeyer), phenolsulphonephthalein was excreted by kidneys in normal amounts This drug evidently has a decided selective action for the kidneys and is not, in any way, affected by concomitant skin lesions Article to be published later

color was obtained through the skin, though the urine both before and after the procedure was heavily pigmented

Indigo carmin was administered by injecting 4 c c of a 4 per cent solution into the gluteus maximus, thirty minutes before sweating The urine showed a deep blue color, indicating a high output of the drug, while the sweat, which was definitely acid, did not show the slightest trace Rosanilin given under similar conditions in another case also failed to be excreted through the skin

Phloridzin was injected in 5 mg doses and readily produced a glycosuria, but in the two cases examined no trace of glucose could be detected in the sweat with Nylander's test

Hexamethylenamin was tried in two cases In one, the patient was given 35 grains in a moderate amount of water within two hours of sweating, and in the other the patient was on a daily régime of 45 grains Both cases showed a definite excretion through the skin, which may explain the pruritus occasionally present in patients on a urotropin régime

SUMMARY

The facts shown by this study of sweat from eighteen normal cases may be summarized as follows

1 Uric acid and diastase are apparently constant constituents of normal sweat

2 Body-weight does not necessarily effect proportionate change in the amount of sweat eliminated nor in the total nitrogen content

3 No definite relationship exists between the amount of nitrogen eliminated and an increase in body temperature during the procedure

4 The relationships between the different forms of nitrogen in sweat and urine are entirely different The concentration of urea in sweat is from three to ten times as high as that in the blood, but only one-tenth (1/10) the concentration of urea in the urine

5 The sweat-glands have the power of concentrating ammonia, and the ratio of ammonia nitrogen to the total nitrogen is considerably higher than that in the urine

6 Uric acid occurs in sweat in much smaller amounts than in either blood or urine, the concentration being about one-twentieth (1/20) that in blood and about one five-hundredth (1/500) that in urine

7 Diastatic activity, though slightly variable in sweat, shows a mean value, lower than that in either blood or urine

8 The commoner dye-stuffs used for colorimetric estimation of kidney function are not eliminated through the skin

9 Phloridzin injections are followed by a negative sugar reaction in sweat, although the reaction is positive in urine, from the same case

CONCLUSIONS

It has been demonstrated that no apparent relationship exists between the chlorids, sulphates and phosphates excreted in urine and in sweat, and it has been repeatedly shown that the kidney concentrates urea, ammonia, uric acid and diastase Our study has demonstrated that the glands of the skin concentrate urea and ammonia, but that the diastatic activity is less than that in either blood or urine, and that uric acid is present in less concentration than in the blood The relative concentrating power of the skin and kidney for urea and ammonia are very different, though in both cases the percentage values are higher than those for blood These facts, in conjunction with the fact that the kidneys show a decided selective action for many dyestuffs, while on the other hand, mercury seems to be more readily excreted through the skin, lead us to conclude that, under normal conditions, the skin and kidneys show a distinct selective action The bearing of this on sweat-baths as a therapeutic measure in cases of uremia and nephritis is evident The various substances which are producing toxemia in a given case may be among those which the kidney can concentrate, while in sweat they are found in less concentrated form than in the blood-stream In the former case, sweating would not be indicated Hence, certain reservations should be made on the efficacy of such procedures as a routine method of treatment and much further work remains to be done on the relation existing between skin and kidneys in pathological conditions, accompanied by cumulative phenomena in the blood-stream, with especial regard to obstruction in the urinary tract

We are greatly indebted to Dr H H Young for the clinical material on which this study is based

212 Professional Building—227 W Monroe Street

STATUS LYMPHATICUS IN ADULTS, ITS CLINICAL DIAGNOSIS AND IMPORTANCE*

HAVEN EMERSON, M D
NEW YORK

At the present time our knowledge of the condition recognized as status lymphaticus, or as it is more commonly called by German writers — status thymicolymphaticus, is not based on etiology, but on physical characteristics

It is not essential to the pursuit of clinical studies that we should have a theory of the disturbed physiology, which determines the abnormal physical individual, nor yet await the complete understanding of the separate or mutual functions of the various secreting tissues, before we admit the relation of the clinical findings to health and disease What is important for the internist, and for the eugenist, is the recognition of a type of individual whose physical peculiarities seem to bear an important relation to his mortality, in certain infectious diseases, and to his susceptibility to some of the deteriorating influences which modern society tolerates or suffers from This recognition of a type of person, who may not appear less healthy or less capable than the rest of society, is at present based wholly on physical examination, one may almost say, on inspection alone

Inspection of the human body provides us with physical signs of health or disease on which we rely The value of physical signs, in relation to health and disease, depends on the constancy of association of the observed facts during life with a definite series of findings after death

If the physical examination reveal constantly certain variations from the usual, or the accepted normal, and if the post-mortem examination reveal a constant series of gross and microscopical abnormalities in a tissue or groups of tissues, then where these two sets of observations are noted in a high per cent of cases among those dying of certain of the infectious diseases, we may presume that there is some causal relation between the physical findings and the mortality of such individuals

From the time when Paltauf (1889-90) pointed out the pathological symptom complex of status thymicolymphaticus, pathologists have more and more generally verified his observations, and in some instances have added to the data he gave in defining the condition found post mortem

* Submitted for publication, Oct 1, 1913
* Read before the Section in Medicine, Seventeenth International Medical Congress, London, Aug 8, 1913

When Neusser (1911) gave an equally clear description of the clinical picture of status thymicolymphaticus, an impetus was given to observations by the internists, to test their skill in establishing a reliable clinical diagnosis, with the possibility of its bearing on the prognosis or treatment of various diseases

Having watched the cases of meningitis, typhoid fever, and septic infections in the general medical wards of Bellevue Hospital die apparently in a high percentage of cases when they showed the physical marks of status lymphaticus, and having noted at the autopsy table the constancy of pathological findings which confirmed the clinical description of such cases, I wished to test the reliability of the various criteria given for the recognition of a characteristic or classical case of status lymphaticus

The material on which this study was based was at my disposal while attending the alcoholic wards at Bellevue Hospital in New York These wards receive patients suffering from the toxic effects of alcohol and of other narcotic drugs, cases of attempted suicide and sick prisoners Among the admissions are often to be found border-line cases, which, on further study, prove to be psychopathic cases rather than toxic individuals of normal mentality One thousand cases were studied, five hundred consecutive male admissions in July and August, 1912, and an equal number in January and February, 1913

The record of each patient consisted of the following points

Age, country of birth, cephalic index, based on the occipitofrontal and biparietal diameters, the color and physical qualities of the hair of the head, the amount of hair on chin, the upper lip, on the chest in front, in the axillae, and the amount and distribution of pubic hair, the amount of faucial, lingual and pharyngeal lymphatic tissue, observed by inspection and digital palpation, the presence of palpable cervical, axillary and inguinal lymph-nodes, the shape and proportions of the neck, thorax, abdomen thighs and upper arms, the details of the external genitals, the general nutrition, the presence of congenital anomalies, the clinical diagnosis

Of the 1,000 cases examined, 780 were without any stigmata or physical characteristics which would mark them as abnormal

Two hundred and twenty showed most if not all, of the physical attributes sufficient to identify them as cases of status lymphaticus

Analysis of the records of these two groups shows certain facts

As to age it was found that 18 63 per cent of the status cases were under 30 years, while but 9 66 per cent of the normal cases were under 30 years Above the age of 30 the distribution of the status and of the normal cases showed such slight discrepancies as to lead to no conclusions The patients' ages ranged from 19 to 84 years

As to nationality or race the variations in the two classes were negligible Eighteen nationalities were represented

As to cephalic index, if we use the term dolichocephalic to represent an index of 80 or less and brachycephalic for an index of over 80, we find that the cases of status are almost equally divided, while the normal cases show a preponderance of 7 per cent of dolichocephalics

Status Lymphaticus, Per Cent	Normal Cases, Per Cent
Dolichocephalic, 49 14	53 63
Brachycephalic, 50 86	46 37

In noting the color, texture (i e, fine or coarse), luster, curliness or straightness of the hair of the head, no differences of any importance were noted

The beard was noted as being scanty in 9 87 per cent of normal cases, but scanty or wholly lacking in 57 22 per cent of the status cases

Similarly it was found that the mustache was scanty in but 7 94 per cent of the normal cases, but scanty or lacking in 55 90 per cent of the status cases

Sternal or anterior thoracic hair was found to be scanty in 41 15 per cent of the normal cases, and scanty or wholly lacking in 89 54 per cent of the status cases

The axillary hair was scanty in 13 45 per cent of the normal cases, but scanty or absent in 65 45 per cent of the status cases

The pubic hair was found to be of the feminine type (i e, arranged transversely and not rising toward the umbilicus, but ceasing abruptly above the pubic region) in 13 20 per cent of the normals, and in 95 90 per cent of the status cases

The faucial, lingual and pharyngeal tonsillar tissue was found increased in amount in the normal cases in 6 66 per cent, 26 02 per cent and 4 48 per cent, respectively, and in the status cases in 7 27 per cent, 23 18 per cent and 0 90 per cent, respectively, not notable differences except in the case of the pharyngeal tonsils Even though the pharyngeal tonsil seems to be enlarged five times as often among status cases as in normal men, the percentage of incidence is too low to use for diagnostic purposes

The case of the superficial lymph-nodes is somewhat similar, for although the cervical, axillary and inguinal nodes are found palpable in somewhat higher proportion of cases in the status individuals than among normals, the occurrence of such palpable glands in adults is so common as to allow of no diagnostic application of this point

In normal cases the cervical, axillary and inguinal glands were palpable in 15 25 per cent, 33 33 per cent and 53 71 per cent respectively, and among status cases in 25 90 per cent, 41 81 per cent and 55 90 per cent, respectively

The neck was noted as long and slender in 32 per cent of normals and in 36 81 per cent of status cases—an unimportant difference

The thorax was found long and narrow in 27 94 per cent of the normal cases and in 44 09 per cent of the status cases

The contour of the abdomen as to its roundness or flatness showed no difference worth noting between the two classes

The thighs were noted as rounded in 30 52 per cent of the normal cases and arched in 13 97 per cent of the normal cases, while in 75 46 per cent of the status cases they were rounded and in 50 per cent were arched These terms, rounded and arched, are used to express the impression made by the difference between the masculine and feminine types of thigh, the rounded as contrasted with the flat thigh being characteristic of feminine physique, and an outward and forward bowing or arching being a feminine attribute as distinguished from the straighter masculine thigh

In the case of the upper arm it was found that 25 76 per cent of normal cases were described as having rounded arms, while among the status cases there were 75 90 per cent with this feminine characteristic

Among the normal cases the penis was noted as small in 22 56 per cent, while this was the case among the status cases in 51 83 per cent

The glans penis was noted as small in normal cases in 26 03 per cent and in status cases in 54 54 per cent

The shape of the glans was described as pointed or acorn-shaped in normal cases in 32 05 per cent and in status cases in 61 31 per cent

There were no undescended testicles among the normal cases, but there were six cases or 2 7 per cent found with undescended testicles among the status cases, four with both undescended, two with one undescended

The size of the testicles was noted as small in 24 74 per cent of normal cases, and among the status cases in 45 90 per cent

As to general nutrition there were no differences of any moment

Congenital anomalies were found in 0 25 per cent of the 780 normal cases and in 3 63 per cent of the 220 status cases

Complicating medical affections, in addition to drug addiction, were found in 3 58 per cent of the so-called normal cases and in 10 40 per cent of the status cases, 94 4 per cent of all cases were admitted for alcoholic intoxication

To summarize the data obtained from these examinations, it may be said that 22 per cent of the particular class of men representing chiefly the alcohol and narcotic drug habitues, show sufficiently striking variations from the usual masculine physical habit to be classed as cases of status lymphaticus, that, of the points usually noted in arriving at a diagnosis, those found to be constant enough to be entitled to considera-

tion are, scantiness or absence of hair on lip, chin, and in sternal and axillary regions, together with the feminine type of distribution of pubic hair with or without scantiness of pubic hair, a tendency to narrowness and length in the thorax, a noticeable roundness and arching of the thighs and roundness of the upper arms, small size of penis, smallness and pointed shape of glans penis and smallness of the testicles

In addition, there is a physical character noted in a very large proportion of the cases, and present almost invariably when the points above summarized are also present, namely, a particularly velvety, or fine and delicate texture of the skin, which is seen and felt to be almost wholly free from the usual short hairs, to be found commonly, for instance, on the thigh in men For lack of such terms as would accurately designate this quality of the skin, I have not made a record of its appearance in the cases above reported, but I wish to call attention to this as an important diagnostic point

Of the points described by others as commonly noted in cases of status which I find of little or no value in arriving at a diagnosis are, the physical characters of the hair of the head, which is supposed to be commonly coarse and straight in status cases, the faucial, lingual and pharyngeal lymph tissue, which is supposed to be commonly increased, the superficial lymph-nodes which are supposed to be felt in a high proportion of status cases, the slender columnar neck and the rounded feminine abdomen which are reported as being usual in status

In addition, there is a point, described by European observers, which I have so regularly failed to make on physical examination of status cases, and which has so little foundation in the experience of the pathologists at Bellevue Hospital, that I have not made it a part of my records, namely, the spleen The spleen is supposed to be large in status This I do not find to be the case

I am permitted by the Director of the Pathological Laboratory to quote from the forthcoming hospital report the results of autopsy records of status since 1905

Of the 3 600 autopsies, 288, or 8 per cent, were cases of status lymphaticus

Of these 288 cases, 29 66 per cent were recessive cases, as shown by microscopical as well as gross study of the lymphatic tissues of the body, and 70 33 per cent were active cases Twenty-six of the 288 cases (or 9 02 per cent) were not associated with other demonstrable lesions as a cause of death

Two hundred and sixty-two (or 91 08 per cent) were associated with other lesions

Of these 262 cases, 242 were associated with infectious diseases

Epidemic, purulent, or tuberculous meningitis

Typhoid fever
Lobar pneumonia
Acute and chronic pulmonary, and general miliary tuberculosis
Acute and chronic cardiac infections
Septic infections of a variety of origins

It is to be particularly noted that of the autopsied cases of typhoid fever 23 07 per cent were status Of autopsied cases of acute infective endocarditis 19 51 per cent were status, and of the autopsied cases of epidemic cerebrospinal meningitis 48 27 per cent, while of the autopsied cases of lobar pneumonia 2 96 per cent were status, of tuberculous meningitis 3 80 per cent were status, of acute and chronic pulmonary or general miliary tuberculosis 4 50 per cent were status

In arriving at a pathological diagnosis at post mortem the essential points to be observed in addition to those accessible to the clinician during life, are the gross and microscopic appearance of the blood-vessels, particularly the aorta, which is small, soft and elastic, the intestinal lymph adenoid tissues, the Peyer's patches and follicles, and the mesenteric lymph-nodes which are uniformly found enlarged, either a hyperplasia of the lymph follicles of the spleen in active cases, or fibroid tissue tufts which have replaced previous hyperplasia of the lymph follicles in the recessive cases, enlargement of the thymus gland out of proportion to the age of the subject in active cases

It is true that percussion and possibly Roentgen ray examination will, in the hands of a few, elicit the presence of an enlarged thymus in children, but the likelihood of this decreases with every decade of the patient's age above the first By deep digital palpation in the episternal notch the normal aorta can usually be appreciated at each systolic impulse, and this is rarely the case in status cases, owing to the small size of the aorta, but this is of little reliability in arriving at a diagnosis

For the present it seems to me that a diagnosis of stat[illegible]phaticus is justified, when we find, in the case of a man, a decided s[illegible]ntiness of hair on chin and upper lip, scanty axillary and sternal hair, scanty or feminine distribution of pubic hair, the slender thorax, rounded contour of upper arms and thighs with an arching of the latter, hypoplastic external genitals, particularly if associated with cryptorchismus, and a delicate velvety skin The diagnosis is further confirmed if we find hyperplasia of the lymph tissue of nose, throat and tongue and an increase in the palpable cervical and axillary lymph-nodes

If we admit the justice of such a diagnosis, because of the confirmation of a sufficient number of cases verified post mortem, of what service will this prove in the practice of medicine?

The obvious and justifiable conclusion at present is that such individuals are more susceptible to the dangers of infectious diseases than

are normal people To avoid for such people by all precautions, especially by artificial immunization, as for typhoid, diphtheria and meningitis, every chance of infection, is our evident duty They are poor risks in an epidemic, and the prognosis for them, when they are infected, is more serious than for normal men As they have passed the particular dangers of their state during childhood, by especial care or good fortune, so, during maturity, they are among those who cannot be expected to survive, as well as normal men, extreme fatigues, shocks, loss of blood, abuse of narcotic drugs or surgical procedures Those physicians whose hospital experience is mainly with people of the upper walks of life, will undoubtedly find that the incidence of status lymphaticus is far below the figures I have presented here for alcoholic patients And in the general medical wards of Bellevue Hospital, which receives almost exclusively the pauper class, the incidence is less than a quarter of that found among alcoholics

Among psychopathic cases, particularly those who suffer acute alienation in the first three decades, the incidence of status is, I believe, even higher than among the cases I have reported above

It should by no means be understood from this that all cases of status lymphaticus are undesirables, socially, as they may be physically less capable in the struggle for existence than their fellows, for it is in the experience of all who observe these cases that there are among them leaders in scientific and artistic professions, and men of the highest standards of character

For the identification of status lymphaticus among women we rely on the peculiar character of the skin of the body and extremities, the scantiness of the axillary hair pad, the scantiness of pubic and perineal hair, hypoplasia of the genital apparatus, and particularly slender thorax and extremities Some women of decided status conformity have a marked growth of hair on the face and upper lip As has been pointed out by Paltauf, Bartel and others, the status cases among women are in a high percentage of cases subject to the dangers of pregnancy and the puerperium With this addition, what has been said of male status cases applies equally to women

While we await the embryological, or physiological, explanation for this particular physical habit (lymphatismus, hypoplastic constitution, constitutional inferiority, status thymicolymphaticus, or status lymphaticus), there is much which the clinician is equipped and particularly fitted to discover as to the relation between status lymphaticus and the incidence and course of various pathological states

In closing, let me gratefully acknowledge my debt to Dr Charles Norris, Director of the Pathological Laboratory at Bellevue Hospital, who was the first in the United States to recognize the clinical picture of status lymphaticus in adults, and place its diagnosis on a sound basis for clinician and pathologist.

Without his experience and records of the past seven years I could not have prepared this paper

120 East Sixty-Second Street

BIBLIOGRAPHY

The list of references appended to the monograph of Bartel and Bauer in 1912, and the classical articles by Paltauf and v Neusser make a further bibliography at this time unnecessary

Bartel and Bauer Status Thymico Lymphaticus u Status Hypoplasticus, Leipzig, 1912

v Neusser Zur Diagnose des Status Thymico Lymphaticus, Wien, 1911

Paltauf, A Ueber die Beziehung des Thymus zum plotzlichen Tod, Wien klin Wchnschr, 1889, No 46, and 1890, No 9

The Archives of Internal Medicine

Vol XIII FEBRUARY, 1914 No 2

STUDIES ON THE CIRCULATION IN MAN

XI. THE BLOOD-FLOW IN THE HANDS AND FEET IN CERTAIN DISEASED CONDITIONS OF THE VESSELS OR OF THEIR VENOUS MECHANISM *

G N STEWART, M D
CLEVELAND

I ARTERIOSCLEROSIS

It need scarcely be pointed out that several of the patients in the previous paper suffered from arteriosclerosis, in addition to valvular lesions of the heart or myocardial changes In this section a few cases are brought together in which arteriosclerosis was pronounced and other lesions if present did not dominate the clinical picture As a general result of the observations, it may be stated that in marked arteriosclerosis the flow in the hands is always smaller and the vasomotor reflexes weaker than in normal persons Vasodilatation is easier to obtain by the application of warmth to the contralateral hand than vasoconstriction by the application of cold

One of the purest cases of marked arteriosclerosis is that of Meyer G, a man aged 39, without detectable cardiac involvement The flow was only 2 88 grams for the right hand and 3 46 grams for the left hand per 100 c c of hand per minute, with room temperature 22 C An increase to 3 96 grams in the right was occasioned by immersion of the left hand in warm water, and the flow only fell to 3 52 grams when the left hand was subsequently put into cold water It must not be forgotten, however, in connection with the small flow in this case that the man was a peddler accustomed to expose his hands in the open air in winter Also he was a dispensary patient, and came from his work on a day in February [1]

Mever G, Hebrew peddler, aged 39 height 5 feet 6½ inches, was admitted at the dispensary Sept 3, 1910, with marked arteriosclerosis There was great thickening of all accessible arteries The heart examination was negative in result He complains of having had pain in the stomach during the past year A test-meal showed free hydrochloric acid No masses are palpable in the abdomen He attributes his condition to strain in pushing heavy loads on his hand-cart The hand flow was measured Feb 3, 1911 He still complains of pain

* From the H K Cushing Laboratory of Experimental Medicine, Western Reserve University

1 See Paper IX of this Series, THE ARCHIVES INT MED, 1913, XII, 678

in the epigastrium On emptying capillaries on the back of the hand by pressure the color returns very slowly Pulse, 72 Mouth temperature, 36 7 C Systolic pressure in the right brachial, 110 Hands put into bath at 2 49 p m , into calorimeters at 2 59½ [2] Volume of right hand in calorimeter, 525 c c , of left hand, 525 c c He is right handed His hands are large (Table 1)

Another outside worker (John O'R), a street laborer aged 60, with a diagnosis of arteriosclerosis and myocarditis, had a flow of 5 49 grams per 100 c c per minute in the right hand and 5 44 grams in the left hand with room temperature 23 C The contralateral vasoconstriction to cold

TABLE 1.—CALORIMETRIC MEASUREMENTS IN CASE OF MEYER G

Time	Right	Left	Notes	Time	Right	Notes
2 58½	29 74	29 81		3 28	29 98	
3 01	29 69	29 75		3 29	30 00	
3 02	29 70	29 74	Room temp 22 2 C	3 30	30 02	
3 03	29 70	29 74		3 31	30 07	At 3 31 left hand put in water at 7 2 C
3 04	29 70	29 74				
3 05	29 70	29 75		3 32	30 09	
3 06	29 70	29 75		3 33	30 11	
3 07	29 69	29 76		3 34	30 13	
3 08	29 69	29 75		3 35	30 16	
3 09	29 69	29 75		3 36	30 18	
3 10	29 69	29 78	Room temp 22 3 C	3 37	30 20	Room temp 21 9 C
3 11	29 71	29 80		3 38	30 21	
3 12	29 72	29 81		3 39	30 22	
3 13	29 73	29 82		3 40	30 23	
3 14	29 75	29 85		3 41	30 25	
3 15	29 77	29 89		3 42	30 27	At 3 42 left hand dried and wrapped in warm cloth
3 16	29 78	29 90				
3 17½	29 80	29 93		3 43	30 29	
3 19	29 82	29 97		3 44	30 31	
3 20	29 83	30 00		3 45	30 33	
3 21	29 86	30 01	Room temp 21 7 C	3 46	30 36	Room temp 22 C
3 22	29 88	30 03		3 47	30 39	
3 23		30 08	At 3 23 left hand put in water at 43 C	3 48	30 42	
				3 49	30 45	Room temp 22 2 C
3 24	29 90			3 50	30 48	At 3 50 hand out of calor
3 25	29 92			3 51		Temp of L is now 29 75 C
3 26	29 94		Room temp 21 6 C	4 16	30 13	
3 27	29 96			4 17		Temp of L is now 29 48

was slight, the flow in the right hand diminishing from 5 49 grams to 4 78 grams, but warmth gave a fair vasodilatation after the usual preliminary vasoconstriction The protocol has already been published [3]

Charles S , a man aged 69, with sclerosed vessels, high blood-pressure and enlarged cardiac dulness, but no murmurs, had a flow in the right hand of only 5 25 grams per 100 c c per minute and in the left a flow of 5 01 grams, with the high room temperature of 28 3 C The response

2 In all the observations unless otherwise stated the quantity of water in each hand calorimeter was 3,015 c c

3 Cleveland Med Jour , 1910, x, 385

of the vessels to reflex impulses from the contralateral hand was very poor, cold producing a small diminution in the flow, warmth no response whatever beyond the initial flow

Charles S, a hammer man, aged 69, was admitted to Lakeside Hospital, June 26, 1911 He had gonorrhea forty-five years ago, never any chancre He has been a heavy coffee and tea-drinker except in the last four or five years He seldom drank whisky or beer, and smokes little Six years ago following an attack of pneumonia he ate a heavy meal and immediately felt short of breath, dizzy and faint He has had dizzy spells ever since The attacks of dyspnea are worse at night and come on suddenly Lately there has been swelling of the feet and ankles There is Cheyne-Stokes respiration with periods of complete apnea lasting forty seconds The anteroposterior diameter of the thorax is increased There is difficulty in expelling air from the lungs The cardiac dulness extends from 7 cm to the right of the midsternal line in the fourth interspace to 14 5

TABLE 2.—Calorimetric Measurements in Case of Charles S

Time	Right	Left	Time	Right	Notes
2 45		30 65	3 00	30 95	
2 46	30 61		3 01	30 99	Room temp 28 3 C
2 47		30 68	3 02	31 005	
2 48	30 67		3 03	31 02	
2 49		30 73	3 04	31 035	3 04 L hand put in water at 43 C
2 50	30 73		3 05	31 055	Room temp 28 3 C
2 51		30 77	3 06	31 09	
2 52	30 76		3 07	31 11	
2 53		30 825	3 08	31 125	
2 54	30 825		3 09	31 135	
2 55		30 87	3 10	31 155	
2 56	30 88		3 11	31 20	At 3 11 hand out of calorimeter
2 57	30 905		3 12½		Temp of L is now 30 76 C
2 58	30 92		3 29	31 08	Room temp 28 15 C
2 59	30 94				

Cooling of calorimeters, R, 0 12 degree in eighteen minutes, L, 0 11 C degree in seventeen and one half minutes Volume of right hand in calorimeter 437 c c, of left hand 410 c c

cm to the left of the midsternal line in the fifth interspace The first sound at the apex is very tympanitic in quality with a slightly roughened element in it Second sound normal, no murmurs or adventitious sounds The blood-vessels are sclerotic Pulse regular in rate and rhythm The ascent is slow, no dicrotism Blood-pressure systolic, 180, diastolic, 120 Some edema of the skin of the genitalia and of the ankles is present The skin is cool and dry He has been on cardiac diet and treated with atropin and nitroglycerin Hemoglobin, 95 per cent Diagnosis, arteriosclerosis and myocarditis He was discharged "improved," July 19, 1911

The blood flow in the hand was examined July 13, 1911 Mouth temperature, 36 3 C, pulse (sitting), 80 Hands put into bath at 2 32 p m, into calorimeters at 2 44 At 2 55 p m left hand put into water at 8 5 C Cooling of calorimeters, R, 0 12 degrees in eighteen minutes, L, 0 11 degree in seventeen and one-half minutes Volume of right hand in calorimeters, 437 c c, of left hand, 410 c c

The last case to be referred to under this heading is that of Fred S, a man 58 years old, with pronounced arteriosclerosis of the vessels of the limbs, mitral insufficiency and pulmonary emphysema The initial flow was 5 76 grams per 100 c c per minute for the right hand and 5 80 grams for the left hand, with an average room temperature of 23 8 C Immersion of the left hand in warm water caused no change in the flow in the right for three minutes, an unusually sluggish reaction, but for

TABLE 3.—CALORIMETRIC MEASUREMENTS IN CASE OF FRED S

Time	Right	Left	Room Temp C	Time	Right	Room Temp C	Notes
2 32½	31 09	31 07		3 00	31 78		
2 35	31 06	31 05	23 4	3 01	31 81		
2 36	31 07	31 05		3 02	31 855	24 0	
2 37	31 08	31 06	23 6	3 03	31 895		At 3 03 left hand put in water at 8 C He feels it very cold and withdraws hand intermittently
2 38	31 095	31 07		3 04	31 91		
2 39	31 11	31 08		3 05	31 92	23 9	
2 40	31 125	31 10	24 0	3 06	31 925		
2 42	31 17	31 15		3 07	31 95		
2 43	31 195	31 165		3 08	31 975	24 0	
2 44	31 22	31 185		3 09	31 995		
2 45	31 25	31 22	23 8	3 10	32 02		
2 46	31 29	31 26		3 11	32 04	24 0	
2 47	31 32	31 29		3 12	32 06		At 3 12 left hand put in water at 38 5 C
2 48	31 36	31 34	23 9	3 13	32 085		
2 49	31 395	31 37		3 14	32 11		
2 50	31 425	31 40		3 15	32 13	24 2	
2 51	31 45		23 9	3 16	32 165		
2 52	31 48			3 17	32 195		
2 53	31 505			3 18	32 22	24 2	
2 54	31 54		24 0	3 19	32 24		
2 55	31 59			3 20	32 27		
2 56	31 62			3 21	32 295		
2 57	31 66		23 9	3 22	32 32		At 3 22 hand out of calor
2 58	31 70			3 23			3 23 Temp of L is 31 00 C
2 59	31 74		24 0	3 45	32 02		3 45 Temp of L is 30 79 C

the next ten minutes of immersion of the left hand in warm water the flow in the right rose to 7 69 grams per 100 c c per minute Immersion of the left hand in cold water caused a reduction of the flow in the right to 3 65 grams per 100 c c per minute for the first three minutes, the flow then rising to 5 54 grams per 100 c c per minute for the next six minutes of immersion When the left hand was again put into the warm water, there was only a small increase in the flow in the right (to 6 46 grams per 100 c c per minute for ten minutes' immersion)

Fred S, a laborer, aged 58, was admitted to the City Hospital April 27, 1913, with marked arteriosclerosis, pulmonary emphysema and mitral insufficiency

He complains of shortness of breath (asthma) which began in October last He has not been able to work since He says the condition seems stationary, neither improving nor growing worse When he gets chilled an attack is brought on The point of maximal cardiac impulse is 5 cm outside the left nipple line The greatest breadth of cardiac dulness extends at the sixth rib to the anterior axillary line, right border, right sternal margin Roughened first sound at apex followed by systolic murmur, which is lost as the base is approached Pulse fairly regular rhythm and rate, fair volume Liver enlarged Urine contains no albumin A few hyaline and finely granular casts Hot baths seemed to have a good effect on him

The blood-flow in the hands was examined May 8, 1913 Hands in bath at 2 22½ p m , in calorimeters at 2 33½ At 2 50 p m left hand put into water at 43 C (Table 3)

Cooling of calorimeters, R , 0 30 degree in twenty-three minutes, L , 0 61 degree in fifty-five minutes Pulse (sitting) 116 Volume of right hand 497 c c , of left hand 484 c c Rectal temperature 37 4 C Water equivalent of calorimeters with contents, R , 3,492, L , 3,482 Blood-pressure left arm, systolic 126, 63 (sound gone) Another observation Systolic 122, 101 (sudden diminution of sound), 63 (sound gone)

II AORTIC ANEURYSM

If we leave out of account John M , a dispensary case who came in with cold hands on a winter day and could not wait for sufficient time to warm up and whose measured flow (3 96 grams per 100 c c per minute for the right hand) is therefore certainly too low,[4] a point of some consequence comes out in Table 19 in which the results are summarized, that is, that for the age of the patient and the temperature of the room the hand flow in these cases of thoracic aneurysm are either of the normal order of magnitude or at least not so conspicuously deficient as in those cases dealt with in Paper X, in which the stress of the pathological change falls on the myocardium rather than on the blood-vessels

John M , an iron-molder, aged 32 years, weight 140 pounds, height 5 feet, 3½ inches, was admitted at the dispensary suffering from aortic aneurysm as shown by the Roentgen ray He complains of pain over the sternum Systolic blood-pressure, right arm 155, left arm 150 Blood-flow in the hands examined Feb 13, 1911 Pulse (sitting) 72 Mouth temperature 37 0 C Room temperature 22 2 C His hands were cold when he came in Right hand in bath at 2 56 p m , in calorimeter at 3 07 and out of calorimeters at 3 36 At 3 25 left hand put into water at 8 5 C

It is not, of course, suggested that in some of the cases of aneurysm deterioration and disordered function of the myocardium or crippling of the valves may not be of graver import than the aneurysm itself But so far as the aneurysm, or rather the underlying condition which has occasioned the aneurysm has not led to deterioration of the heart, there is no reason in the mechanics of the circulation why the blood-flow through

4 The fact that immersion of the left hand in cold water caused only a very slight diminution in the flow in the right while immersion of the left hand in warm water increased the flow decidedly (to 5 5 grams) indicates that the vasoconstriction produced by the exposure of the hand to the cold was initially considerable

the hand should suffer This is true even where the form of the pulse-wave may be greatly distorted by its passage through the aneurysmal sac Of course, a sufficient degree of pressure of the sac on a subclavian artery or vein may diminish the flow in the corresponding hand The great difference in the flow in the two hands in Paul K may be accounted for by the pressure of the aneurysm on the left subclavian artery The flow in his hands (for the last fifteen minutes in the calorimeters) was 7 64 grams for the right per 100 c c per minute and 5 9 grams for the left, with room temperature 22 6 C (a ratio of 1 3 1) For the right hand this flow is not markedly subnormal for his age The diagnosis was auricular fibrillation with mitral insufficiency (and stenosis), well compensated at the time of the blood-flow examination and aortic aneurysm There

TABLE 4.—CALORIMETRIC MEASUREMENTS IN CASE OF JOHN M

Time	Right	Time	Right	Time	Right
3 06	29 15	3 19	29 28	3 29	29 47
3 10*	29 13	3 20	29 30	3 30†	29 48
3 11	29 14	3 21	29 32	3 31	29 50
3 12	29 14	3 22	29 33	3 32	29 51
3 13	29 16	3 23	29 36	3 33	29 54
3 14	29 19	3 24	29 38	3 34‡	29 58
3 15	29 21	3 25	29 40	3 35	29 62
3 16	29 23	3 26	29 42	3 36	29 67
3 17	29 24	3 27	29 425	3 53	29 49
3 18	29 27	3 28	29 44	3 55	29 47

* Room 22 0 C † At 3 30 left hand dried and wrapped up ‡ Room 22 4 C
Volume of right hand 380 c c

is no evidence that the aneurysm, unless by its mechanical pressure, caused any change in the flow

Paul K, a baker, 57 years old, was admitted to the City Hospital Feb 21, 1912 There is a history of lues and of frequent acute attacks of articular rheumatism A history of skin rash was obtained He had gonorrhea ten or twelve times His present illness began three weeks before admission He had an attack of rheumatism at the time and began to be short of breath His eyes have been swollen for three days There is bulging over the heart The thorax is barrel shaped, and many dry râles can be heard A loud systolic murmur can be heard over the apex, the heart is very irregular Not all the heart-beats are felt at the wrist Blood pressure is 110 to 120 The Roentgen ray shows a marked prominence of the aortic arch on the left side and heart enlarged He was put on digitalis The pulse rate, which was 132 on the morning after admission, fell rapidly During March and April it varied between 75 and 90, his clinical condition improved and he was allowed to leave his bed and walk about the ward

The blood-flow in the hands was examined April 24, 1912 Hands in bath at 1 32 p m, in calorimeters at 1 42½, and out of calorimeters at 2 06 (Table 5)

Cooling of calorimeters in ten minutes, R, 0 13 degree, L, 0 12 degree Volume of right hand 454 c c, of left hand 451 c c He works mainly with his left hand although he is right-handed Rectal temperature 38 C Water equivalent of calorimeters with contents, R, 3,458, L, 3,456 After the examination it was noticed that the heart-rate was much increased (to 140 counted with a stethoscope) and that it was more irregular than before

In Joseph C, a dispensary patient aged 50, with marked arteriosclerosis, a high systolic pressure (160 mm) and distinctly dilated aortic arch, the flow was 6 69 grams for the right hand and 6 66 grams for the left, with room temperature 21 5 C Considering the amount of arteriosclerosis and that in arteriosclerosis, as already stated, the hand flow has a tendency to be diminished, there is no evidence in this case that the presence of the aneurysm as such has reduced the flow

Joseph C, an iron molder, aged 50, height 5 feet, 9½ inches, was admitted to the dispensary Jan 30, 1911 He has been ill for six or seven years, during which time he has done no work Six years ago he lost consciousness and after that his left arm was paralyzed The grip of the left hand is weak, too weak, he says, to grasp objects, but on testing this the difference between the hands is not great He gets very dizzy at times There is much arteriosclerosis He

TABLE 5—Calorimetric Measurements in Case of Paul K

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
1 42	31 13	31 29		1 56	31 40	31 465	
1 44	31 07	31 21		1 57	31 45	31 50	
1 45	31 08	31 21	22 8	1 58	31 49	31 525	22 6
1 46	31 095	31 225		1 59	31 51	31 53	
1 47	31 11	31 23		2 00	31 56	31 58	
1 48	31 13	31 25	22 8	2 01	31 605	31 61	
1 49	31 165	31 29		2 02	31 65	31 635	
1 50	31 19	31 31		2 03	31 70	31 67	
1 51	31 21	31 32		2 04	31 75	31 71	22 6
1 52	31 25	31 35		2 05	31 795	31 75	
1 53	31 29	31 38	22 9	2 06	31 83	31 76	
1 54	31 32	31 41		2 16	31 70	31 64	
1 55	31 37	31 43					

suffers from pain in the head, also in the breast and in the left hand The Roentgen ray shows a definite dilatation of the arch of the aorta. Wassermann reaction negative on Feb 6, 1911

The hand flow was examined on Jan 31, 1911 Hands in bath at 2 52 p m, in calorimeters at 3 05, and out of calorimeters at 3 20, 3,056 c c of water in each calorimeter Pulse (sitting) 88 Mouth temperature 37 2 C (Table 6)

Cooling of calorimeters, R, 0 19 degree, and L 0 16 degree in fourteen minutes Volume of right hand 505 c c, of left 480 c c Blood-pressure, systolic in right brachial 160, in left brachial 164

In William B, a man aged 53, suffering from thoracic aneurysm, the flow in the right hand was 8 38 grams per 100 c c per minute and in the left 7 33 grams with room temperature 20 8 C (for fourteen minutes

in calorimeters) The systolic pressure was very high (205 mm) He was still taking nitroglycerin at the time of the blood-flow examination

A second examination made at the dispensary nearly four weeks later showed a flow of 10 23 grams per 100 c c per minute for the right and 9 47 grams for the left hand, with room temperature 22 2 C These flows are certainly not small for a man of his age and with sclerosed arteries The inequality in the flow in the two hands is no greater than may be seen in normal persons, and it will be observed that the ratio between the flows in the two hands is the same at the two examinations (1 1 08)

TABLE 6.—CALORIMETRIC MEASUREMENTS IN CASE OF JOSEPH C

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
3 04	29 13	29 12	20 3	3 16	29 55	29 51	
3 06	29 11	29.10		3 17	29 61	29 53	21 5
3 07	29 14	29 11		3 18	29 67	29 62	
3 08	29 18	29 12	21 5	3 19	29 72	29 67	
3 09	29 20	29 14		3 20	29 78	29 71	
3 10	29 25	29 19		3 32	29 61	29 58	
3 11	29 29	29 22		3 34	29 59	29 55	
3 12	29 34	29 31					
3 13	29 39	29 33	21 4				
3 14	29 45	29 39					
3 15	29 51	29 43					

TABLE 7.—CALORIMETRIC MEASUREMENTS IN CASE OF WILLIAM B FIRST EXAMINATION

Time	Right	Left	Time	Right	Left
2 37	28 38	28 54	2 48	28 70	28 82
2 40	28 34	28 53	2 51	28 90	29 00
2 42	28 39	28 58	2 53	29 04	29 11
2 43	28 43	28 61	2 55	29 20	29 22
2 45	28 56	28 70	2 56	29 27	29 28
2 46	28 61	28 75	3 12	29 09	29 10

William B a man aged 53, height 5 feet, 2 inches, weight 152 pounds, had an aneurysm of the thoracic aorta involving the innominate also to some extent After being under observation at the dispensary for a considerable time he was admitted to Lakeside Hospital where he was under treatment for a month (digitalis and nitroglycerin) His hand flow was examined at the time of his discharge from the hospital He felt much better than when he entered, on Dec 22, 1910 He has no cough but there is a history of cyanosis, although there is no cyanosis now He says his hands always feel rather cool, especially when his heart is bad

Hands in bath at 2 26½ p m, in calorimeters at 2 38½, and out of calorimeters at 2 56, 3,050 c c of water in each calorimeter Room temperature 20 4 C

Room temperature at end of observations 21 2 C Volume of right hand in calorimeter 410 c c, of left hand 390 c c Cooling of calorimeters in sixteen minutes 0 18 C degree Pulse (sitting) 84 Mouth temperature 37 1 C Water equivalent of calorimeters with contents, R, 3,458, L, 3,442 (Table 7)

The second examination was on Jan 17, 1911 He says he feels about the same as at the first examination, only the shortness of breath on exertion is greater Systolic blood-pressure 210 Hands put into bath at 3 09 p m, in calorimeters at 3 21, and out of calorimeters at 3 37, 3,050 c c of water in each calorimeter Room temperature 22 2 C.

TABLE 8—Calorimetric Measurements in Case of William B Second Examination

Time	Right	Left	Time	Right	Left
3 19	30 70	30 49	3 31	31 13	30 86
3 23	30 69	30 48	3 32	31 19	30 91
3 25	30 79	30 57	3 33	31 23	30 94
3 26	30 86	30 62	3 34	31 29	31 00
3 27	30 92	30 67	3 35	31 34	31 07
3 28	30 97	30 71	3 36	31 39	31 11
3 29	31 02	30 77	3 37	31 44	31 16
3 30	31 07	30 81	4 00	31 12	30 85

Cooling of calorimeters in twenty-three minutes, R, 0 32 C degree, L, 0 31 C degree Volume of right hand in calorimeter 435 c c, of left 410 c c Mouth temperature 36 9 C Water equivalent of calorimeters with contents, R, 3,478, L, 3,458 Pulse (sitting) 88

In Richard B, a man aged 38, with a large aortic aneurysm extending, as shown by the Roentgen-ray, to a considerable distance to the right of the sternum, there was a decided deficiency in the flow of the left hand as compared with the right (10 91 grams per 100 c c per minute for the right, 8 15 grams for the left, a ratio of 1 33 1) For the room temperature of 28 C, 10 9 grams is not a large flow, but neither is it decidedly small The vasomotor reflexes from the right to the left hand were normal in direction, and not strikingly deficient in intensity, showing that the chief afferent path in the vasomotor reaction to warmth or cold (the nerves of warmth and cold sensation) have not appreciably suffered by stagnation of the circulation due to the pressure of the aneurysm on the right subclavian vein Now, when pressure is maintained on the arm at a level somewhat below the systolic arterial pressure by means of the cuff of a sphygmomanometer the warmth sensation suffers early, so that it is impossible to appreciate the contact of a warm tube, while the sensation of pain is at this stage less affected As has been pointed out in Paper X, a certain amount of venous distention, provided that the arterial pressure is correspondingly increased, may even be associated with an

increased flow, because of the increased cross-section of the smaller vessels So far as the arterial path to the right arm in Richard B is concerned, the conditions would favor a greater flow in the right hand, for as shown at the necropsy the aneurysm provided a much wider entrance than normal for the blood going to the right subclavian and possibly a reservoir which helped to maintain the flow during diastole

Richard B, a colored gardener, aged 38, height 5 feet, 7½ inches, was admitted to Lakeside Hospital May 22, 1911, with an aneurysm diagnosed as aortic He complains of hoarseness during the past two weeks and vomiting during the past week, also cough He admits lues There is a tender pulsating mass in the suprasternal notch with fulness in the right supraclavicular region Heart 3 cm to left of nipple A distant systolic murmur at the supraclavicular notch The aortic second is slightly accentuated There is distinct diastolic shock, unilateral (right) distention of veins, and clubbing of fingers The Roentgen ray shows an enormous shadow upward and to the right The patient continued in the hospital until his death, Aug 22, 1911 There was no right radial pulse for the last week of life Blood examination on May 23, 1911, showed hemoglobin 95 per cent, leukocytes 5,000 June 1 the Wassermann test was strongly positive The necropsy showed a saccular, spherical aneurysm of the innominate artery 3 to 4 inches in diameter The arch of the aorta was not involved except for the somewhat enlarged ring at the beginning of the innominate opening into the sac

Hand flow examined May 23, 1911 Pulse (sitting) 100 Mouth temperature 37 1 C Hands put into bath at 2 44 p m, into calorimeters at 2 54½ At 3 08 p m right hand put into water at 8 1 C

TABLE 9.—CALORIMETRIC MEASUREMENTS IN CASE OF RICHARD B

Time	Right	Left	Room Temp C	Time	Left	Room Temp C	Notes
2 54	30 43	30 38		3 13	31 225		
2 55	30 475	30 42	27 7	3 14	31 28		
2 56	30 53	30 43		3 15	31 325	28 0	
2 57	30 595	30 46		3 16	31 36		At 3 16 put right hand water at 43 C
2 58	30 695	30 525		3 17	31 38		
2 59	30 78	30 60		3 18	31 415		
3 00	30 86	30 63		3 19	31 46		
3 01	30 935	30 69	27 9	3 20	31 525	28 3	
3 02	31 02	30 74		3 21	31 58		
3 03	31 12	30 825		3 22	31 625	28 2	
3 04	31 20			3 23	31 67		
3 05	31 295	30 93		3 24	31 725		
3 06	31 34	30 98		3 25	31 80		
3 07	31 395	31 02		3 26	31 85	28 2	Hand out of calorimeter
3 08	31 46	31 035		3 41	31 75	28 0	Temp of R is now 31 27
3 09		31 07	28 0				
3 10		31 10					
3 11		31 125					
3 12		31 155					

Cooling of calorimeters, R, 0 19 degree in thirty-three minutes, L, 0 10 C degree in fifteen minutes Volume of right hand 495 c c, of left 437 c c There is an obvious difference in the size of the two hands He is right handed

The next case, that of Samuel S , a man 31 years old, illustrates the fact that even very marked differences in the form of the radial pulse on the two sides, differences perfectly evident both to the finger and on the sphygmogram, may be associated with a comparatively small difference in the flow in the two hands The right radial showed a typical pulse of aortic insufficiency with abrupt ascent to a sharp apex and abrupt fall, while the left radial gave a sphymogram with a rounded apex The blood-pressure in the two brachials was also quite different, the systolic pressure being 157 in the right and 118 in the left The physical signs of aortic insufficiency were present

The blood-flow measurement showed a difference no greater than may be seen in normal persons between the two hands (11 11 grams per 100 c c per minute for the right and 10 32 grams for the left, with room temperature 22 2 C), a perfectly normal flow for a man of this age The conclusion was drawn that the aneurysm in distorting the form of the pulse-wave did not interfere materially with the free passage of blood through the left subclavian artery The man died the same night about five hours after the examination by a rupture of the aneurysm into the left bronchus It is worthy of remark that five hours before his death the blood-flow in his hands, which no doubt in this case was an index of the output of his heart, was quite normal He was to die, not because his heart was failing, but because the wall of the aneurysmal sac had been thinned to the bursting point A very different tale would have been told by the hand flow in a case of impending heart-failure as in Stephen F , or Albert H (Paper X), or Anthony P

Samuel S , a colored music teacher, aged 31, was admitted to the City Hospital Dec 7, 1912, with thoracic aneurysm He is a well-developed man 5 feet, 10½ inches tall, weight 160 pounds He has had spells of intense dyspnea for the past six weeks He has been continually getting worse to the present time, is much cyanosed, and suffers much from cough which he has had from the age of 16 He has smoked cigarettes to excess, and also has used a good deal of alcohol He denies lues although he admits the possibility of it

The left pupil is smaller than the right and does not react to light or accommodation There is diffuse pulsation of the lower third of the neck Tracheal tug is present The left side of the thorax moves less than the right The percussion note is dull over the entire left side [compression of the left lung by the aneurism(?)] Over the left lung the breath-sounds are very faint, distant and bronchial in character The cardiac dulness at the level of the second rib extends three fingers' breadth to the right of the right sternal border and the same distance to the left of the left sternal border In the fourth interpsace it extends from two fingers' breadth to the right of the right sternal border to the left midaxillary line The apex beat is visible and palpable in the sixth interspace, two fingers' breadth outside the nipple line Systolic and presystolic thrills are palpable over the apex Loud booming systolic and diastolic murmurs are heard at apex Loud presystolic murmur followed by systolic murmur is heard over aortic and pulmonic areas The sternum rises fully a half-inch with each systole The radial pulse is regular, rhythmical, asymmetrical, the sphygmogram of the left radial having a rounded apex, and that of the right a sharp (aortic insufficiency) apex The pulse is synchronous in the two radials and

of good volume and tension The difference in the form of the pulse wave is very evident to the finger

The blood flow in the hand was measured Dec 10, 1912 Hands in bath at 1 50 p m, in calorimeters at 1 59½, and out of calorimeters at 2 13 p m

TABLE 10.—CALORIMETRIC MEASUREMENTS IN CASE OF SAMUEL S

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
1 59	31 40	31 43		2 07	32 11	32 13	22 25
2 00	31 435	31 49	22 4	2 08	32 195	32 20	
2 01	31 55	31 58	22 4	2 09	32 27	32 265	22 2
2 02	31 64	31 68		2 10	32 35	32 36	22 1
2 03	31 74	31 78	22 3	2 11	32 395	32 42	
2 04	31 85	31 88		2 12	32 47	32 465	22 3
2 05	31 94	31 95		2 13	32 53	32 53	
2 06	32 01	32 055	22 3	2 41	32 17	32 17	

Cooling of calorimeters in twenty eight minutes, 0 36 degree Volume of right hand 580 c c, of left hand 603 c c Water equivalent of calorimeters with contents, R, 3,559, L, 3,577 Rectal temperature 38 4 C Blood-pressure left arm, systolic 118, 58 (sound suddenly diminished), right arm, systolic 157, 61 (sound abruptly diminished) Another observation on the right arm gave identical results Three days previously, according to another observer, the pressures were 160 (systolic), 45 (change of sound) in the right, and 130, 55 in the left brachial

A necropsy was performed by Dr A A Johnson, Pathologist at the City Hospital, to whom I am indebted for the following details Heart, left ventricle dilated moderately, average thickness 16 to 18 mm Other cavities normal The segments of all the valves except the aortic are normal and the orifices are not dilated The aortic ring in the formaldehyd preserved specimen measured 8 cm The aortic valve segments are slightly thickened and curled along their free edges The openings of the coronary arteries are free and of the usual size. The aorta immediately above the aortic ring is smooth and free from thickening Opening from the anterior aspect of the transverse arch is a large sacular aneurysm adherent to the sternum The sac is oval, 10 by 8 cm in diameter The walls are thin (2 to 4 mm) and are quite free from fibrin The opening from the aorta into the sac measures 5 by 3 5 cm The innominate opens by a somewhat dilated mouth posterior and external to the opening into the aneurysm and situated opposite the middle of the latter opening The left common carotid and subclavian arteries come off from the aorta about 1 cm beyond the upper border of the opening between the aorta and the aneurysm Rupture in the sac on the inferior surface has irregular edges and measures 3 by 1 mm, leading into the left bronchus 1 cm from the bifurcation

It will be seen that there was no stenosis of the orifice of the left subclavian, but its origin was situated distal to the orifice of the aneurysmal sac, while the origin of the innominate was directly opposite the center of that orifice The direction of the opening of the innominate was such that the blood coming from the heart would pass straight into it all the more as the opening of the innominate was somewhat dilated This would account for the abrupt rise in the sphygmographic curve and

the high maximum pressure in the right arm The sudden fall in the sphygmogram if partly due to actual regurgitation through the aortic orifice, was probably due still more to the effect of rapid expansion of the thin aneurysmal sac, which after closure of the aortic valve would still go on yielding and so exert a suction action, especially on the orifice of the innominate placed directly opposite it On the other hand, the action of the large aneurysmal sac lying proximal to the left subclavian would be to damp the pulse-wave in that artery and delay the arrival of the crest This would not necessarily affect the total flow, the pulsatory element in which is measured not by the absolute height of the crest, but by the area enclosed between the sphygmogram and the line of diastolic pressure [5]

The last case included in the group of aneurysms (that of John McL) is the only one which shows a notably small hand flow It is placed in this group merely to emphasize the point that in drawing any general deductions from such measurements the proper classification of the cases is of great importance

John McL, a plasterer, aged 50, height 5 feet, 11½ inches, was admitted to the City Hospital July 6, 1912, with general edema (in face, hands, arms, legs and feet), also cyanosis in the lips and fingers He complains of "kidney trouble" He looks anemic The heart shows an impure first sound at the apex The aortic second is greatly accentuated, the blood-pressure is high (systolic 200) The abdomen is distended with free fluid Albumin and casts were present in the urine all along On July 11 about 7 liters of fluid was removed from the abdomen There was no detectable fluid in the thoracic cavity A diagnosis of hepatic cirrhosis was made Aug 19, 7,080 c c of fluid was removed from the abdomen

On Aug 27, 1912, the hand and foot flows were estimated and found exceedingly small (0 41 gram per 100 c c per minute for the right hand and 0 57 gram for the left hand, with room temperature 22 2 C , 0 17 gram per 100 c c per minute for the right foot and 0.14 gram for the left foot, with room temperature 21 2 C)

Aug 28, 1912, 1,965 c c of liquid was taken from the abdominal cavity at 1 30 p m and the hand and foot flows were again measured They were larger than on the previous day, but still quite small (1 11 grams per 100 c c per minute for a period of seventeen minutes for the right hand and 1 18 grams for the left hand, with room temperature 21 1 C , 0 66 gram per 100 c c per minute for the right foot for the last ten minutes of a period beginning twenty minutes after insertion of the feet into the calorimeters, and 0 65 gram for the left foot) The ratio between the flows in the feet and hands (1 1 75) is near enough the normal to permit the conclusion that the small flow in both has a common cause The presence of an aneurysm of the aorta was not

5 Paper V of this series, THE ARCHIVES INT MED , 1912, ix, 706

diagnosed during life The small blood-flow in the hands has certainly nothing to do with the existence of the aneurysm It is without doubt dependent on the cardiorenal, or cardiorenalhepatic affection, that is to say, on such factors as the vasoconstriction of which the high blood-pressure is one expression, on the coincident anemia, and on the crippling of the heart muscle The small flow in the feet may also depend in part on the mechanical hindrance imposed by the fluid in the peritoneal cavity or in the connective tissue of the legs But if this were an important factor the ratio of hand to foot flow would be disturbed

The patient died Dec 27, 1912 The necropsy showed general peritonitis, aneurysm of the descending thoracic aorta, cloudy swelling and parenchymatous change in the kidneys The pericardium was adherent to the left pleura, but not to the epicardium The heart was pale (weight 300 grams), with some dilatation and hypertrophy of the left ventricle The mitral orifice was dilated and the edges of the mitral valve were thickened and puckered The edges of the aortic valve were thickened and slightly curled The sinuses of Valsalva were dilated Many sclerotic plaques were about the coronary openings The right coronary vessels were not patent The left coronary vessels were free but sclerosed The aortic ring was much dilated, 10 5 cm in circumference The whole intima of the aorta was very much thickened Here and there were areas of sclerosis The openings of the large vessels were dilated Eight centimeters beyond this there was an opening on the left posterior wall of the aorta 2 cm in diameter, opening into a sac 4 cm in diameter The body of the fifth thoracic vertebra had been slightly eroded Beyond this the thoracic aorta was much thickened in irregular areas About 1,000 c c of straw colored fluid was in the left pleural cavity The abdomen contained about 10 liters of cloudy fluid with many shreds of fibrin The visceral and the parietal peritoneum was dark in color and covered with exudate The intestines were adherent The liver showed no scarring, weight 1,660 grams In the kidneys the cortex was not sharply differentiated from the medulla The whole kidney was dark in appearance, with cloudy swelling to parenchymatous change For these details I am obliged to Dr A A Johnson

III MISCELLANEOUS VASCULAR CONDITIONS INCLUDING VASCULAR NEUROSES

Among these are three cases of Raynaud's disease

Margaret H , aged 15, suffering from Raynaud's disease in the fingers, had a flow in the right hand of 5 33 grams per 100 c c of hand per minute and 6 67 grams in the left, with room temperature 23 6 C The protocol has already been published [6] The considerable inequality in the flow in the two hands is probably due to a functional vasomotor difference When there are no anatomical reasons for such a difference in flow it either disappears at subsequent examinations or is reversed as regards the two hands In cases in which a marked difference is obtained in the two hands in several examinations, the deficiency being always in the same hand and the ratio of the flows in the two hands not changing greatly, there is reason to believe, in the absence of obvious anatomical differences, that the difference in flow may depend on pathological

6 Heart, 1911, iii, 79

changes in the thorax or in the arm or hand itself which have not yet definitely disclosed themselves by other symptoms

The flow in Margaret H is distinctly subnormal, which corresponds with the fact that her hands were cold to the touch, although she did not have an attack during the examination The relatively small flow may be related to the tendency to dyspnea which she states has existed for some years The most striking result of the examination was the intense reflex vasoconstriction produced in the left hand when the right was put into cold water, the flow being reduced from 6 67 grams to 2 97 grams per 100 c c per minute Although intense, the vasoconstriction was transient, lasting only for two minutes and then giving place to distinct vasodilatation, the flow increasing to 8 28 grams in the left hand, while the right continued in the cold water Immersion of the right hand in warm water produced no further increase, but rather a decrease The vasomotor mechanism of the hand seems, therefore, to respond with abnormal readiness to vasoconstrictor influences and with less than normal readiness to vasodilator influences The short duration of the intense crossed reflex vasoconstriction relates the case to those of early peripheral neuritis,[7] and it is of interest, in this connection, that neuritic changes may be associated with the Raynaud's syndrome

The second case of Raynaud's disease was that of Mrs W G, aged 28, who was seen April 24, 1911 About one and one-half years previously, after a sleigh ride, she noticed that the fingers of both hands became cold, "white like those of a dead person" and numb Sometimes the fingers became purple All the phalanges were involved She feels sure they were not frost-bitten Since that time she has had many attacks, each lasting usually five to ten minutes, but occasionally almost an hour The attack generally begins in the thumb Putting the hands into cold water does not necessarily bring on an attack A cold full bath is more likely to do so The thumbs are apt to become numb if she holds anything in her hands for any length of time Long before this condition came on her arms and hands used to go to sleep a great deal even when she was not lying on them Also for as long as she can remember small injuries to the skin of the hands or arms healed badly She pointed out the scar of a small burn on the left hand which was still raised above the surface although otherwise healed It was a most trifling burn she said Even a little scratch leaves a scar She showed various marks of old injuries on the arm She has been fighting the condition by going out to walk without gloves, continuing to walk with her hands exposed even when they became numb, and thought that some time she improved it in this way, her hands afterwards becoming warm Her general method has been to immerse them in warm water on coming in The attacks are apt to be induced by a damp day, even if not cold The hands to day feel markedly cold to the touch, not only the fingers, but the hands midway to the wrists The day was not cold and she was wearing kid gloves Her arms are mottled with purple spots She has no trouble in the feet, nose, or elsewhere Her general health is satisfactory, but there is some shortness of breath on exertion She is fat and well nourished, and the arms and hands are quite plump She suffers much from nervousness

7 Paper XII of this series

The blood-flow in the hands was examined April 24, 1911 Pulse 108 (sitting) Mouth temperature 37 55 C Hands put into bath at 10 10 a m At 10 15 it was seen that the left arm was getting purple, the right arm remained unchanged She said the left hand felt colder than the right, and that the left arm and hand felt as if swollen Hands put into calorimeters at 10 20 a m

TABLE 11.—CALORIMETRIC MEASUREMENTS IN CASE OF MRS W G

Time	Right	Left	Notes	Time	Right	Notes
10 19	30 125	30 13		10 43	30 57	
10 24	30 11	30 13		10 44	30 60	
10 25	30 12	30 15	Room temp 25 3 C	10 45	30 62	
10 27	30 17	30 19		10 46	30 67	Room temp 24 9 C
10 28	30 20	30 22		10 47	30 70	At 10 47 left hand put in water at 12 C
10 29	30 22	30 24				
10 30	30 25	30 26		10 48	30 71	
10 31	30 26	30 26		10 49	30 72	
10 32	30 27	30 27		10 50	30 73	
10 33	30 29	30 31		10 51	30 75	
10 34	30 31	30 32	At 10 34 put left hand in water at 43 C	10 52	30 77	
				10 53	30 79	
10 35	30 33					
10 36	30 36		Room temp 27 C Face and ears red She says the left hand feels swollen greatly	10 54	30 81	At 10 54 dried and wrapped left hand It is red and is not specially cold to touch Room temp 27 4 C
10 37	30 38			10 55	30 83	
10 38	30 40			10 56	30 86	
10 39	30 42		Room Temp 25 3 C	10 57	30 89	
10 40	30 47			10 58	30 92	Hand out of calorimeter at 10 58
10 41	30 50					
10 42	30 54			10 59		10 59 temp of L is 30 30 C
				11 24	30 74	11 24 temp of L is 30 06 C Room temp 25 8 C

Cooling of calorimeters, R, 0 18 C degree in twenty six minutes, L, 0 14 degree in twenty-five minutes Volume of right hand 391 c c, of left hand 375 c c Water equivalent of calorimeters with contents, R, 3,408, L, 3,395 The left hand may be swollen a little, as her ring, which had been removed, now goes on with difficulty, yet the subjective feeling of extreme swelling which she had is obviously no index of the actual swelling

The small initial flow in this patient (3 62 grams per 100 c c per minute for the right hand and 3 44 grams for the left, with the relatively high room temperature of 25 3 C) is considerably more striking than in the case of Margaret H This agrees well with the longer duration and greater development of the condition in Mrs W G Immersion of the left hand in warm water caused a fair and persistent increase in the flow in the right (to 5 16 grams), although the absolute flow was still small Subsequent immersion of the left hand in cold water caused a strong vasoconstriction in the right hand, diminution of the flow to 2 46 grams per 100 c c per minute for the first three minutes and 3 93 grams for the last four minutes Although the temperature of the cold water was

purposely made higher than usual (12 C instead of 8 C), she complained greatly of the cold and withdrew her hand intermittently This was seen also in the other cases, and the hypersensitiveness of the afferent receptors to cold as gauged by the sensation elicited has its parallel in their hypersensitiveness to cold as components of the reflex vasomotor arc

The third case of Raynaud's disease (Mrs C B) illustrates the condition when it has existed for a long time and has gone on to gangrene The age of the patient was 29 Not only was the local condition more severe than in the last case, but the general physical condition was worse The hand flow for a period of sixteen minutes before the vasomotor reaction was tested was 3 23 grams per 100 c c per minute for the right hand and 4 29 grams for the left For the last six minutes of this period the flows worked out at 3 65 grams and 3 80 grams for the right and left hands, respectively The reflex reaction to warmth was fair (the flow in the right hand increasing for the first two minutes of immersion of the left in warm water to 6 55 grams per 100 c c per minute), but it was transient, and for the last ten minutes of the period the flow was only 4 30 grams per 100 c c per minute The reflex reaction to cold was also anomalous, possibly because of the existence at this stage of the disease of considerable anatomical changes in the vessel

Mrs C B , aged 24, was admitted to the Lakeside Dispensary June 1, 1908, suffering from Raynaud's disease She was treated with nitroglycerin without benefit April 23, 1913, on account of coughs, sweats, and general weakness she was referred to the Medical Department of the Dispensary when the following data were elicited Both hands are affected The condition was first noted one day in winter seven years ago, the fingers becoming cold and pale and then getting purple The attacks have been growing steadily worse, especially in the last five years Sometimes small grangrenous areas form quickly at the ends of the fingers and quickly recover, but always with some loss of substance, so that the terminal phalanges of several of the fingers have lost a considerable proportion of their length and most of them have lost their nails On all the fingers the nails are atrophic She has considerable pain in the fingers when they "break out" She has had pain and marked stiffness in the right wrist and right forearm for about two years, and she uses the right hand but little on account of the stiffness of the wrist The skin of the right forearm is somewhat stretched and distinctly glossy in comparison with that of the left She cannot bear to put her hands into cold water She has no such symptoms in her feet, nose or elsewhere Some years ago she had a sore on her ankle, due, as she thinks, to a habit of kicking the ankle with the other foot The sore refused to heal and she was put in hospital for several weeks, during which time her general health and strength improved greatly, and the sore also improved, although it took a long time before it was healed She is pale and thin There is some pigmentation of the skin and some dulness at the apex of the left lung The radial pulse is small

Examination of the blood-flow in the hands was made May 9, 1913 The marked pallor with some lividity and the "stone cold" feeling of the hands especially of the fingers, was striking, although she was wearing gloves The day was rather chilly The terminal phalanx of one finger of the right hand had a small sore on the point of it which she said caused her some pain Pulse (*sitting*) 112 weak Hands in bath at 3 15 p m , in calorimeters at 3 27 Mouth temperature, 37 35 C

TABLE 12.—CALORIMETRIC MEASUREMENTS IN CASE OF MRS C B

Time	Right	Left	Room Temp C	Time	Right	Notes
3 26	31 18	31 17		3 53	31 135	3 52 left hand put in water at 43 C
3 28	31 09	31 13		3 54	31 155	Room 22 8
3 29	31 09	31 13	21 3	3 55	31 16	
3 30	31 08	31 12		3 56	31 17	Room temp 22 9 C
3 31	31 08	31 12	21 5	3 57	31 175	
3 32	31 08	31 11		3 58	31 18	
3 33	31 085	31 11		3 59	31 195	Room temp 22 95 C
3 34	31 08	31 11	21 8	4 00	31 20	
3 35	31 08	31 11		4 01	31 20	
3 36	31 08	31 115		4 02	31 205	
3 37	31 085	31 12	22 0	4 03	31 21	
3 38	31 085	31 12		4 04	31 225	At 4 04 left hand put in water at 12 C
3 39	31 085	31 125		4 05	31 24	She feels it very cold Left hand taken out of water intermittently
3 40	31 09	31 14		4 06	31 25	
3 41	31 085	31 15	22 1	4 07	31 255	Room temp 22 7 C
3 42	31 09	31 16		4 08	31 26	The cold water is now at 13 5 C The left hand is red She says if it had not first been in warm water it would have been purple in the cold water To the touch the left wrist is warm (vasodilatation), much warmer than the right
3 43	31 09	31 17		4 09	31 27	
3 44	31 09	31 18		4 10	31 28	
3 45	31 09	31 195		4 11	31 295	
3 46	31 095	31 20	22 0	4 12	31 305	
3 47	31 10	31 205		4 13	31 34	4 13 hand out calorimeter
3 48	31 10	31 21		4 43	30 96	4 43 temp L 30 58 C
3 49	31 105	31 215				
3 50	31 115	31 22	22 6			
3 51	31 12	31 225				
3 52	31 12	31 23				

Cooling of calorimeters, R, 0 38 C in 30 minutes, L, 0 65 C in 51 minutes Volume of right hand 273 c c, of left 282 c c She is right handed Water equivalent of calorimeters with contents, R, 3 313, L, 3 320

It may be noted that in these three cases of Raynaud's disease there was evidence that a coexisting or preexisting general feebleness of the circulation conspired with the local condition in producing the symptoms The blood-flow measurements suggested that if the general circulatory conditions had been normal the local tendency to vasoconstriction might not have sufficed to reduce the flow to the level at which the symptoms became obtrusive This was the suggestion merely derived

from the three cases studied and it is not claimed that it has a general application At any rate since hand flows habitually as small or smaller are often encountered in cases which never develop Raynaud's disease, the primary factor must be a tendency, whatever its essential cause, to the local diminution in the flow

In a case of "dead fingers" in an elderly man affecting only the right hand, the flows were among the smallest observed in the whole investigation, and this not only in the hand obviously affected, but also in the other The patient had been sitting in a well-warmed room for an hour before the examination, yet the calculated flow for the right hand was only 0 09 gram per 100 c c per minute and for the left 0 25 gram, with room temperature 20 5 C A fair reflex vasodilatation was obtained in the right hand on the immersion of the left in warm water, the flow increasing to 0 5 gram, still of course a very small flow, but relatively a great increase The dead condition of the right hand is therefore probably not due to anatomical change in the vessels, but to a vasoconstriction

The next case in this group is that of Maurice T , a man 41 years old, who had lost a leg through obliterative arteritis

Maurice T , a tailor's cutter, height 5 feet 7 inches, weight about 175 pounds, a well-built man, apparently in perfect health He was seen April 17, 1911 About eight years ago in winter he began to feel the toes of the left foot cold The little toe was first affected, gradually the same condition came on in the other toes After awhile the toes became painful also The whole foot became involved by degrees Then cramp-like pains began to trouble him in the calf of the left leg The trouble was worse in winter Small nodules on the foot and toes became gangrenous Eventually, four years ago, the left leg had to be amputated above the knee The arteries were found extensively affected He never had any pain above the knee About six months ago he noticed that the little toe of his right foot was cold Later on it began to pain him Now the whole right foot is cold to the touch and the pulse in the dorsalis pedis poor The little toe has a raw surface where it is contact with the next toe and the nail is apparently going to come off He knows of no injury to it The pain in the foot is so great that while he is in bed he cannot sleep When he stands the pain disappears, but he has pain also when he walks The arch of the right foot is broken down and he has to wear a support in his shoe There is hyperidrosis of the foot in spite of the fact that it is cold The flow in the hands was examined April 17, 1911 Mouth temperature 36 5 C Hands put into bath at 10 05 a m and into calorimeters at 10 18 Pulse (sitting), 92

At this time the foot calorimeters had not been constructed so that only an examination of the flow in the hands was made This was done with the idea that some information of possible value for a prognosis in regard to the leg condition might be obtained, (1) from the absolute amount of the hand flow as an index of the flow in the periphery in general, (2) from the nature of the reflex vasomotor response as an index of the existence of a tendency to vasomotor spasm, a functional condition which there is good reason to believe may underlie or at least precede in certain cases the development of obliterative arteritis The flow in the

right hand was 4 87 grams per 100 c c per minute and in the left 6 67 grams, with room temperature 25 7 C, a distinctly subnormal flow Immersion of the right hand in cold water caused one of the most intense reflex vasoconstrictions in the contra-lateral hand which has been witnessed in the whole course of these observations, the flow declining to 2 59 grams per 100 c c per minute for the first five minutes and only reaching 5 59 grams after a further immersion in the cold water of four

TABLE 13.—CALORIMETRIC MEASUREMENTS IN CASE OF MAURICE T

Time	Right	Left	Notes	Time	Left	Notes
10 17½	29 57	29 58		10 38	30 24	Room temperature 25 5 C
10 20	29 58	29 60				
10 21	29 60	29 62		10 39	30 29	At 10 39 put right hand in water at 43°
10 22	29 62	29 65	Room temperature 25 7 C			
10 23	29 66	29 70		10 40	30 31	
10 24	29 70	29 76		10 41	30 34	Room temperature 25 0 C
10 25	29 74	29 82				
10 26	29 78	29 86	Room temperature 25 6 C	10 42	30 38	Room temperature 25 1 C
10 27	29 81	29 92		10 43	30 45	
10 28	29 85	29 97	Room temperature, 26 C Ears red	10 44	30 52	Room temperature 25 2 C
				10 45	30 60	Ears still red
10 29	29 89	30 02		10 46	30 65	
10 29½			Right hand put in water at 9 C	10 47	30 72	Room temperature 25 2 C
10 30		30 08		10 48	30 80	At 10 48 dried and wrapped right hand
10 31		30 09				
10 32		30 11	The cold water is now 10 6 C	10 49	30 82	
10 33		30 12	He feels the cold water extremely	10 50	30 88	Room temperature 25 2 C
10 34		30 14	Room temperature 26 6 C	10 51	30 94	
				10 52	31 00	Room temperature 25 3 C
10 35		30 15				
10 36		30 19		10 53	31 07	
10 37		30 22		10 54	31 13	Hand out of cal
				11 09		Temp of room 29 65 C
				11 09½	31 01	

Volume of right hand, 470 c c , of left hand, 466 c c He is right handed

minutes The reflex to warmth is also well marked, the flow in the left hand eventually rising to 10 96 grams The general indication of the observations as regards the question propounded was that a vasomotor instability with a tendency to excessive vasoconstriction in the extremities might be the basis for the symptoms in the foot The inequality in the flows in the two hands corroborates the idea of some degree of vasomotor abnormality Anatomical changes in the nerves were of course not excluded Unfortunately, the man soon after removed to a distant part

of the country, and up to the present it has not proved possible to learn the further history of the case

In the case of Anthony P , a man 36 years old, with gangrene of the fingers and toes, the question arose whether the condition depended on a vasomotor neurosis

Anthony P , a Polish laborer, aged 36 years, height 5 feet 8 inches, weight 150 pounds, was admitted to City Hospital, Oct 9, 1912, complaining of sore fingers and toes He froze his feet last winter There is no history of frost-bite of the fingers He had numbness of the fingers a year ago Owing to the condition of his fingers he was forced to quit work about three weeks before admission On admission the right hand showed beginning gangrene at the tip of the thumb, loss of the distal half of the nail of the middle finger, the remainder being undermined and loss of the entire nail of the ring-finger The left hand showed purplish discoloration of the thumb, the nail being absent, loss of the entire nail of the index finger except the root, and gangrene about the base of the nail of the ring-finger

A blood-flow examination of the hands was made on Oct 11, 1912, with a very warm day (room temperature 27 8 C) The flow came out only 1 22 grams for the right hand, and 1 12 grams for the left per 100 c c per minute, or allowing for the swelling of the left hand, 1 27 grams The pulse-rate was 120 (sitting) The conclusion drawn was that if the small flow depended solely on the local conditions it was unlikely that the constriction of the vessels was purely functional, since the high room temperature would have caused vasodilatation He was in such poor condition that a sufficient examination of the vasomotor reflexes was postponed In the meantime cyanosis of the face and dilatation of the neck veins became prominent and physical examination revealed the signs of serious involvement of the heart (mitral stenosis, aortic insufficiency and signs of impairment of the myocardium) It was no longer possible to draw deductions from the small hand flow, and the slight response to the high external temperature as to the condition of the local circulation, since the state of the heart itself was sufficient to account for the small flow even provided that the cutaneous vasodilatation was the maximum possible with so small a driving power Also fever (his rectal temperature was 39 65 C) is associated with cutaneous vasoconstriction and a small hand and foot flow [8] Had the feet been normal, a comparison of the hand and foot flow might still have yielded information, since the cardiac insufficiency might be expected to diminish the flow in both without affecting the normal ratio between the hand and foot flow, whereas a vascular constriction (vasomotor or anatomical) confined to the hands would be revealed by a disturbance of this ratio in favor of the feet The local conditions, however, soon became a matter of indifference in view of the serious involvement of the heart The patient died October 12

8 Stewart, G N · Paper VIII, Jour Exper Med , 1913, xviii, 372

The necropsy showed the following Left lung infarct in the lower lobe 5 cm in diameter Right lung infarct in upper lobe 2½ cm in diameter, and in lower lobe 5 cm in diameter Heart engorged on the right side and left side dilated, heart-muscle dark and flabby The pericardium contains 100 c c of fluid Aortic valve, left cusp hardened and shortened Mitral admits tip of index-finger Vegetations about the valves Tricuspid, edges of valves thickened, recent vegetations Liver, 1,800 grams, with chronic passive congestion Spleen, weight 140 grams, chronic passive congestion The kidneys show much arterial thickening Blood-vessels radial, practically no arterial thickening The fingers show stasis of blood in the vessels A cross-section of the index-finger shows much perivascular infiltration, but no thickening of the vessel walls Pathological diagnosis, septic endocarditis —(From Dr Johnson's report)

In a case of diabetic gangrene of the feet in a man 63 years old, the flow with a room temperature of 25 6 C was 1 39 grams for the right foot and 1 83 grams for the left, or allowing for the swelling of the latter 1 95 grams An interesting point is that the flow in the foot in which the gangrene had made most progress was greater than in the other foot There is scarcely any question that this is due to inflammatory reaction The diminished flow in the toes actually involved, if the flow is diminished there, would not show in a measurement of the flow through the whole foot The sum of the flows in the feet, although subnormal, is not strikingly deficient, and the ratio of foot to hand flow (1 1 85) falls within the normal range The hand flow was measured on the day preceding the observations on the foot and came out 2 65 grams for the right hand and 3 32 grams for the left per 100 c c per minute, with room temperature 22 0 C In hospital cases presenting no rapid clinical changes, our experience has shown that such observations can safely be compared The fact that the flow both in the hands and feet is decidedly below the normal agrees with the well-known feebleness of the cutaneous circulation in diabetes, and is probably in part due to cardiac changes The vasomotor reflex, when warm water is applied to the contralateral hand, is very poor Whether this indicates the beginning of pathological changes in the hand arteries also cannot of course be definitely stated It is in any case interesting to correlate the enfeeblement of this vasomotor reflex with the enfeeblement or disappearance of skeletal reflexes such as the knee-jerk, which is known to occur in long-standing cases

James P , a teamster 63 years old, was admitted to the City Hospital, Aug 16, 1911, with a small ulcer on the inner surface of the left great toe and a scar in the same position on the right great toe He came for treatment for his foot It was found that the urine contained 4 per cent of sugar with a trace of albumin He was put on a diabetic diet The blood count showed erythrocytes 4,890,000, leukocytes 7,800, hemoglobin 90 per cent April 28, 1912, the percentage of sugar in the urine was reduced to 0 8 April 11, 1912, when the blood-flow in the feet was examined there were small necrotic areas on the great and second toes of the right foot The distal half of the left foot was bluish-red and the foot considerably swollen The toes of the right foot were also bluish-red He says that sometimes the left foot is hot, but when the flow in the feet was examined this was not the case

Feet in bath at 1 21 p m , in calorimeters at 1 33, and out of calorimeters at 2 00, 3,740 c c of water in each calorimeter [9]

Mouth temperature 37 45 C Pulse (sitting), 68

TABLE 14.—Calorimetric Measurements in Case of James P

Time.	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
1 37	30 82	30 76		1 51	30 995	30 97	
1 38	30 82	30 80	25 9	1 52	30 98	31 00	
1 39	30 86	30 82		1 53	31 01	31 01	25 4
1 40	30 875	30 83		1 54	31 02	31 025	
1 41	30 885	30 84		1 55	31 03	31 04	
1 42	30 885	30 855	25 8	1 56	31 035	31 05	
1 43	30 905	30 87		1 57	31 05	31 06	
1 44	30 92	30 88		1 58	31 05	31 08	
1 45	30 935	30 915		1 59	31 06	31 10	
1 46	30 94	30 92		2 00	31 02	31 09	25 4
1 47	30 95	30 935		2 28	30 76		
1 48	30 96	30 95	25 2	2 29		30 75	
1 49	30 975	30 96		2 32	30 71*		
1 50	30 98	30 96		2 33		30 72*	

* After better stirring

Cooling of calorimeters, R , 0 31 degree in thirty-two minutes, L , 0 37 degree in thirty-three minutes Volume of right foot in calorimeter 1,090 c c of left foot 1,175 c c Rectal temperature (taken by himself and doubtless too low as compared with mouth temperature), 37 5 C Water equivalent of calorimeters with contents, R , 4,760, L , 4,820

The next case is remarkable for the fact that the flow in the hands per 100 c c per minute was very much less than in the feet, a quite unusual circumstance

K , a laborer aged 50, was admitted April 15, 1912, to the City Hospital He was much cyanosed on admission, and when the flow in the hands was first examined next day the cyanosis was still well marked in the face, nose, ears and hands, although less pronounced than on admission. There is gangrene of the toes on left foot The pulse can be felt in the dorsalis pedis Blood-pressure 140 systolic, 100 diastolic He had some cough and râles of every description were heard all over the lungs The fingers were markedly clubbed The heart-sounds were weak A soft systolic murmur and reduplicated second sound were heard over the left sternal border, more marked at the base The heart was enlarged The pulse at the wrist is not strong, but by no means so weak as to suggest that the blood-flow in the hands would be found so small as is actually the case

April 16 the flow in the right hand came out only 0 27 gram per 100 c c per minute and in the left hand 0 28 gram, for a period of eighteen minutes in the calorimeters, with room temperature 23 8 C Immersion of the left hand in warm water caused no increase whatever in the flow in the right The very small flow in the hands agreed with the diagnosis

9 For the details of measurements of foot-flow see Paper VII of this series, Jour Exper Med , 1913, *xviii*, 354

of myocarditis It is possible also that a factor in the small flow was nervousness, as this was the first examination, and the patient was a foreigner, speaking no English Still the effect of nervousness on the flow usually passes away more quickly

April 18 he was again examined The cyanosis was now much diminished and the radial pulse was stronger The flow in the right hand was 1 05 grams and in the left 1 43 grams per 100 c c per minute, with room temperature 22 5 C , still quite a small flow, although much greater than at the last examination In the right foot the flow was 2 15 grams per 100 c c per minute, and in the left foot 3 42 grams Immersion of the right foot in cold water was accompanied by a reduction in the flow in the left foot to 2 68 grams, which was only slightly increased (to 2 87 grams) when the right foot was put into warm water

Feet in bath at 2 07 p m , in calorimeters 2 19 p m Patient sitting 3 740 c c water in each calorimeter At 2 45 p m the right foot was put into water at 10 5 C

TABLE 15 —Calorimetric Measurements in Case of K

Time	Right	Left	Room Temp C	Time	Left	Notes
2 22	29 99	29 93		2 46	30 605	
2 23	30 00	29 95	20 5	2 47	30 625	
2 24	30 02	29 99		2 48	30 635	Room temp 20 7 C
2 25	30 03	30 05		2 49	30 655	
2 26	30 045	30 08		2 50	30 665	
2 27	30 07	30 125		2 51	30 70	
2 28	30 08	30 14	20 4	2 52	30 715	
2 29	30 09	30 175		2 53	30 725	
2 30	30 10	30 22		2 54	30 745	At 2 54 right foot put in
2 31	30 11	30 245				water at 42 8 C
2 32	30 125	30 275		2 55	30 76	Room temp , 21 0 C
2 33	30 135	30 30		2 56	30 775	Right big toe painful
2 34	30 145	30 33		2 57	30 80	
2 35	30 17	30 35	20 1	2 58	30 825	
2 36	30 19	30 38		2 59	30 835	
2 38	30 21	30 42		3 00	30 85	
2 39	30 22	30 46		3 01	30 87	
2 40	30 23	30 47		3 02	30 90	Room temp 21 3 C
2 41	30 23	30 50		3 03	30 925	
2 42	30 235	30 52		3 04	30 935	
2 43	30 24	30 535		3 05	30 94	Foot out of calorimeter
2 44	30 26	30 56		3 17	30 75	Room temp 20 9 C
2 45	30 275	30 595		3 18		Temp of R is now 29 79 C

Cooling of calorimeters, R , 0 485 C in thirty three minutes, L , 0 19 C in twelve minutes Rectal temperature 37 6 C Volume of right foot in calorimeter 950 c c , of left 985 c c Water equivalent of calorimeters with contents, R , 4,652, L , 4,679

The question whether the gangrene was going to spread was what chiefly interested the physician in charge of the case The fact that the

flow in the feet was so good was interpreted as favorable for the prognosis, although of course it was impossible to say to what extent the flow in the toes themselves had been interfered with The left foot was somewhat swollen, as shown by the volume measurement Yet it was only a little over 3 per cent larger than the right The greater flow in the left was probably related to a certain degree of inflammatory reaction The foot soon began to heal up But on April 25 the temperature, which had hitherto never surpassed 100 F, began to rise, reaching 104 2 F on April 27 Then it fell and remained below 100 F till his death on May 3

The existence of a relatively good flow in the feet associated with a poor flow in the hands demands some further remark It is probable that the immediate cause of the lesion in the left foot was exposure to cold If so, it is likely that the already existing feebleness of the circulation and the poor response of the vasomotor regulatory mechanism were contributing causes Very likely both feet suffered injury, although it only went on to gangrene in the left The flow in both feet may, therefore, have been increased by the reaction to the injury caused by the freezing, and this in spite of the feeble driving-power of the heart Thus the flow in the feet may have been brought up by the accident of the inflammation approximately to the normal flow, while that in the hands fell far below the normal

Miss W, aged 25, weight 128 pounds, has lost fourteen pounds since her illness began She first noticed pain in the ankles with some swelling in March, 1908 The pain increases as the day goes on, and at about 7 p m it is worse but on lying down she gets relief In the morning her eyes are swollen The wrists also swell when she works much with her arms There is some shortness of breath She must lie down some part of the day if she desires to sit up at night, even to 9 o'clock An eruption consisting of raised red spots comes out periodically on the legs, also, though much less copiously, on the arms There is burning pain and itching in the spots In a day they get brownish, and in two days have disappeared to a great extent, except that a brown stain persists for weeks Several spots 2 cm in diameter which had come out the previous day on her legs were examined The reddish color did not diminish on pressure From her description the spots resemble urticaria at first and then hemorrhage takes place into the wheals On the trunk also are spots which she describes as wheals, which itch and are painful, but the most purpuric looking spots are on the legs On the front of each tibia is a spot with a blackish crust from which she says some matter came out, but generally no liquid escapes from them Her feet and toes are cold and so are her hands especially the terminal phalanges of the fingers Her nails grow irregularly, probably more quickly than before, and are ridged There is distinct defect of sensation in the toes and the adjacent parts of the foot A pin-prick is scarcely felt, and then only as contact She does not feel any tickling sensation when the sole is stroked and there is no plantar reflex Good knee-jerks can be obtained There is a long reddish scar on the back of one finger of the left hand She says she had "blood poisoning" some time ago in a small wound caused by a tack and healing did not occur for eighteen weeks The heart-sounds are clear and unaccompanied by murmurs The urine contains no albumin, casts or sugar Her eye sight is not affected

The blood-flow in the hands was examined May 9, 1911 Pulse 88 Mouth temperature 37 6 C Hands put into bath at 10 14½ p m, and into calorimeters at 10 24

TABLE 16.—CALORIMETRIC MEASUREMENTS IN CASE OF MISS W

Time	Right	Left	Notes	Time	Right	Notes
10 23	30 13	30 08	Room tempera-, 24 0 C	10 43	30 195	Room temperature 24 3 C
10 25	30 11	30 06		10 44	30 20	
10 26	30 10	30 05		10 45	30 215	At 10 45 left hand put in water at 9 1 C
10 27	30 11	30 05				
10 28	30 09	30 04				
10 29	30 10	30 04	Room temperature 24 1 C	10 46	30 215	She feels left hand very uncomfortable.
10 30	30 10	30 04				
10 31	30 10	30 03		10 47	30 22	
10 32	30 12	30 04		10 48	30 225	Room temperature 24 3 C
10 33	30 12	30 035				
10 34	30 125	30 04		10 49	30 225	
10 35	30 13	30 045		10 50	30 23	
10 36	30 145	30 06		10 51	30 23	
10 37	30 16	30 06	At 10 37 left hand put in water at 43 C	10 52	30 235	Room temperature 24 4 C At 10 52 dried and wrapped left hand It is pretty red
10 38	30 165		Room temperature 24 3 C			
10 39	30 17		Left hand is fairly red and the veins of fair size	10 53	30 235	
				10 54	30 24	
				10 55	30 245	Room temperature 24 5 C
10 40	30 17					
10 41	30 18			10 56	30 245	Hand out of calorimeter Feels pins needles in it
10 42	30 185					
				10 56½		Temperature of L, 29 91 (at 10 56½)
				11 26	30 03	

Cooling of calorimeters, R, 0 215 C in thirty minutes, L, 0 15 C in nineteen and one half minutes Volume of right hand in calorimeter 292 c c of left 298 c c She is right-handed

May 25, 1911, the physician in charge of the case reported that since the time of the blood-flow examination she had had rather severe intestinal symptoms and that her temperature rose to 103 F He gave her calcium chlorid and alkalies She is now much better than for a while and the spots have disappeared

The flow in the right hand was 2 16 grams per 100 c c per minute in the left 1 64 grams, with room temperature 24 1 C, a very poor flow for her age Reflex vasodilatation is absent, or quite feeble, the flow in the right hand only rising to 2 38 grams when the left was immersed in warm water Scraping the skin with a blunt point caused no local vasodilatation Although the initial flow was so small a further reflex vasoconstriction, reducing the flow in the right hand to 1 72 grams, was obtained when the left hand was put into cold water No doubt the

coexisting anemia is a factor in reducing the flow through the hands The toxemia (rheumatic perhaps) on which the purpura depends may also depress the flow more directly, either by enfeebling the heart or by vasoconstriction The conclusion deduced from the blood-flow measurements was that the feeble cutaneous flow might be a factor in the condition, and that so far as this went, the indication was that such measures should be adopted as would increase the flow in the skin, including the treatment of such anemia as was present [10]

The last case to be described in this paper is that of Charles H

Charles H, a clerk 42 years old, height 5 feet 10½ inches, weight 140 pounds, was admitted to the City Hospital Nov 23, 1912, with edema of the right foot and leg The swelling began a month ago, commencing at the toes with inflammation, he says, resembling erysipelas A little over a year ago he accidentally injured the right lower leg by a blow with a hammer about midway between knee and ankle over the tibia It healed at the time Afterward a sore opened from which matter was discharged He entered a hospital, where the leg soon healed He has pain

TABLE 17—Calorimetric Measurements in Case of Charles H First Examination

Time	Right	Left	Room Temp C	Time	Right	Left	Room Temp C
3 26	31 43	31 43		3 51	31 15	31 165	
3 29	31 36	31 31	22 7	3 53	31 135	31 18	23 0
3 31	31 33	31 26		3 55	31 125	31 185	23 1
3 33	31 305	31 24	22 8	3 57	31 12	31 19	23 15
3 35	31 28	31 23	23 1	3 59	31 105	31 21	23 1
3 37	31 26	31 195	23 15	4 01	31 09	31 225	
3 39	31 235	31 18		4 03	31 08	31 24	23 0
3 41	31 22	31 17	23 1	4 05	31 07	31 24	
3 43	31 20	31 16		4 07	31 06	31 245	23 0
3 45	31 18	31 155	23 1	4 10	31 01	31 18	
3 47	31 17	31 155	23 0	4 21	30 865	31 04	
3 49	31 16	31 15					

as soon as he commences to walk When he stands up for a few minutes the foot becomes very blue and the small blood-vessels distend The swelling comes on with great rapidity (in ten or fifteen minutes) when he goes about on his feet, and disappears completely when he lies down for a little while There are no varicose veins He does not appreciate warmth or cold, or contact (blunt-point) on the dorsum of the right foot or on the lower third of the right leg A pin-prick is felt as dull contact There is no anesthesia above the level of the scar due to the injury, except for a small tongue just above and continuous with the scar The right sole is very sensitive It began to be hypersensitive just after the foot commenced to swell When he awakes in the morning he has a burning sensation about the toe-nails of the right foot This disappears when he walks about a little When he stood up it was seen that his right leg became very red in the anesthetic region On pressure the red color disappeared He says that when he first came into the hospital the white spot produced on pressure remained for some time, but now it quickly becomes red again When he first came into the hospital

10 Paper VI of this series, Jour Exper Med, 1913, xviii, 13.

the red color which came on when standing soon became pale blue, whereas after a fortnight in the hospital it remains red The legs, however, continue to be cold to the touch He would not know his leg and foot were becoming red if he did not see it He says he cannot keep the right foot warm When the left foot is sweating under the bedclothes and he puts his hand down to feel the right foot it is always cold He looks anemic and feels pretty weak, though his appetite is good He has lost nearly 30 pounds in weight since he had malaria in Texas three years ago The knee jerks and ankle-jerks are somewhat exaggerated, but there is no patellar or ankle clonus Examination of the heart and lungs is negative in result and the heart sounds are clear though very weak and distant The pulse is regular

The flow in the feet was examined Dec 5, 1912, the patient sitting Feet in bath at 3 15 p m, in calorimeters at 3 28, and out of calorimeters at 4 07, 2,775 c c of water in each calorimeter (Table 17)

Cooling of calorimeters in eleven minutes, R, 0 145 degree, L, 0 14 degree Volume of right foot 961 c c, of left 924 c c He has small feet (No 7 shoes) Rectal temperature 37 6 C Pulse (sitting) 100 Water equivalent of calorimeters with contents, R, 3,696, L, 3,668

Second examination Dec 10, 1912 Pulse (sitting) 96 Hands in bath at 3 14 p m, in calorimeters at 3 27 At 3 40 right hand put into water at 42 8 C

When taken out of the cold water the right hand was distinctly redder than the left Cooling of calorimeters, R, 0 505 C in forty one minutes, L, 0 19 C degree in sixteen minutes Volume of right hand in calorimeter 367 c c, of left hand 355 c c He is right-handed Rectal temperature 37 35 C Blood pressure in right arm, systolic 108, 88 (sudden change in sound), 81 (sound gone) The left arm systolic 108 (Table 18)

TABLE 18.—CALORIMETRIC MEASUREMENTS IN CASE OF CHARLES H SECOND EXAMINATION

Time	Right	Left	Room Temp C	Time	Left	Room Temp C	Notes
3 26	31 89	31 85	21 9	3 47	31 90	22 5	
3 28	31 85	31 81	22 0	3 48	31 91	22 4	
3 29	31 85	31 81	22 15	3 49	31 92		
3 30	31 85	31 81	22 15	3 50	31 925	22 8	
3 31	31 855	31 82		3 51	31 93		
3 32	31 86	31 825	22 4	3 52	31 935	22 9	
3 33	31 865	31 83	22 6	3 53	31 94		
3 34	31 87	31 84	22 8	3 54	31 945	22 7	
3 35	31 875	31 84		3 55	31 955		At 3 55 right hand put in water at 8 6 C
3 36	31 88	31 85	22 6	3 56	31 955		
3 37	31 88	31 855	22 5	3 57	31 955		
3 38	31 895	31 86		3 58	31 955		The right hand is red He feels the water very cold
3 39	31 895	31 86		3 59	31 95	23 7	
3 40	31 895	31 865		4 00	31 945		
3 41		31 875	23 0	4 01	31 94	23 5	
3 42		31 875		4 02	31 94		The right hand does not now feel cold at all Still red
3 43		31 875	22 8	4 03	31 94		
3 44		31 88		4 04	31 935	23 7	
3 45		31 885	22 7	4 05	31 93		Hand out of calor at 4 05
3 46		31 89		4 21	31 74		4 21 temp of R is 31 39 C

The distribution of the anesthesia and the history of the case seem to indicate that a nerve injury was the starting point of the vascular condition The flow in the right foot was 0 51 gram per 100 c c per minute and in the left 1 35 grams, with room temperature 23 0 C The flow in the right hand was 3 43 grams, and in the left hand 3 71 grams per 100 c c per minute, with room temperature 22 6 C While, then, the flow in the hands is much below the normal, which corresponds well with the rather feeble action of the heart and with the patient's anemic condition, the flow in the left foot preserves a ratio to that in the hands not far from normal The flow in the right foot, however, is distinctly poor, even in proportion to the poor hand flow The cause accordingly of the small flow in the right foot is not a central one, but is local This agrees well with the patient's observation that he cannot keep the right foot warm, and that the red color which it first exhibits when he stands becomes pale blue The blood-flow measurement corroborates the conclusion that in the right foot the blood is stagnating in the capillaries of the engorged area

SUMMARY

1 In cases with marked arteriosclerosis the blood-flow in the hands is smaller and the contralateral vasomotor reflexes less pronounced than normal

2 In cases of thoracic aneurysm the flow in the hands may be normal Where it is subnormal there is no evidence, apart from a unilateral deficiency in some cases due to the pressure of the aneurysm, that the existence of the aneurysm as such has any influence in diminishing the flow, the diminished flow being associated with coexisting pathological conditions (myocardial changes, valvular lesions)

3 A great difference in the form of the pulse-wave in the two radials associated with thoracic aneurysm does not necessarily indicate a difference in the flow through the hands The hand flows may be identical when the sphygmograms from the radials on the two sides are very different

4 In three cases of Raynaud's disease the hand flows were subnormal even in the absence of an actual attack, in the more advanced cases very much below the normal

5 An abnormally great contralateral reflex vasoconstriction to cold seems to characterize the earlier stages of Raynaud's malady When it has existed for a long time and gangrene has occurred, the vasomotor reactions indicate the existence of anatomical changes in the vessels, which do not permit vasomotor reflexes as intense as at first

TABLE 19.—SUMMARY OF CALORIMETRIC

Case	Age	Date	Pulse Rate	Blood Pressure	Temperature (C) of				Volume of Part in c c	
					Room	Art Blood	Calorimeters			
							Right	Left	Right	Left
Chas S	69	7/ 8/13	80	180, (120)	28 3	36 3	30 70	30 75	437	410
					28 3		30 96			
					28 3		31 12			
Fred S	58	5/ 8/13	116	122, (101)* 63	23 8	36 9	31 28	31 25	497	484
					23 9		31 47			
					23 9		31 70			
					23 9		31 91			
					24 0		31 99			
					24 2		32 19			
Paul K	57	4/24/12	100	115	22 8	37 5	31 14	31 27	454	451
					22 6		31 52	31 54		
Joseph C	50	1/31/11	88	160	21 5	37 2	29 49	29 43	505	480
Wm B	53	12/22/10	84	205	20 8	37 1	28 81	28 97	410	390
		1/17/11	88	210	22 2	36 9	31 07	30 82	435	410
Richard B	38	5/23/11	100		27 9	37 1	31 03	30 75	495	437
					28 0			31 09		
					28 0			31 26		
					28 1			31 41		
					28 2			31 66		
Sam S	31	12/10/12	124	L ,118,(58) R ,157,(61)	22 2	37 9	31 98	32 01	580	603
John McL	50	8/27/12		156, 83	22 2	36 15	30 73	30 72	498	475
					21 2		30 53	30 44	1,697	1,712
		8/28/12	97		21 1	36 7	30 63	30 59	507	454
					20 4		30 87	30 76	1,780	1,749
Mrs W G	28	4/24/11	108		25 3	37 55	30 22	30 24	391	375
					25 1		30 51			
					25 0		30 73			
							30 77			
					2 66		30 87			
Mrs C B	29	5/ 9/13	112		21 7	37 35	31 09	31 10	273	282
					22 3		31 10	31 27		
					22 3		31 11	31 21		
					22 8		31 14			
					22 9		31 19			
					22 8		31 24			
					22 7		31 25			
					22 7		31 30			
Maurice T	41	4/17/11	92		25 7	36 5	29 84	29 76	470	466
					26 3			30 11		
					26 6			30 22		
					25 0			30 34		
					25 2			30 59		
Anthony P	36	10/11/12	120		27 8	39 15	31 90	31 92	406	460
James P	63	4/10/12			22 0	37 05	30 51	30 59	438	414
					22 0			30 78		
		4/11/12	68		25 6	37 35	30 92	30 93	1,090	1,175
K	50	4/16/12	116	140, 100	23 8	36 7	30 55	30 65	352	310
					24 0		30 44			
		4/18/12			22 5	37 1	29 55	29 72	313	304
					20 3	37 0	30 13	30 26	950	985
					20 7			30 67		
					21 1			30 84		
Miss W	25	5/ 9/11	88		24 1	37 6	30 13	30 05	292	298
					24 3		30 19			
					24 3		30 23			
					24 4		30 24			
Chas H	42	12/ 5/12	100		23 0	37 0	31 11	31 20	961	924
		12/10/12	96	108, (88) 81	22 6	36 8	31 87	31 84	367	355
					22 9			31 87		
					22 7			31 92		
					23 6			31 93		

* As in the other papers, the numbers in brackets in this column give the pressure at which the sound had disappeared

The condensed results of three of the cases referred to in this paper (Meyer, G, J O'R and this series (Heart, 1911, iii, 33)

at Given Off in Gram Calories			Blood-Flow in Gm Per Min		Flow Per 100 c c of Part Per Min		Notes
ht	Left	In Mins	Right	Left	Right	Left	
0	822	8	23 07	20 57	5 25	5 01	Arteriosclerosis
3		8	18 81		4 30		Left hand in water at 8 5 C
4		7	22 18		5 08		Left hand in water at 43 C
9	1,428	10	28 64	28 08	5 76	5 80	Arteriosclerosis
0		3	27 96		5 62		Left hand in water at 43 C
0		10	38 24		7 69		Left hand still in water at 43 C
4		3	18 18		3 65		Left hand in water at 8c
1		6	27 57		5 54		Left hand still in water at 8 c
2		10	32 13		6 46		Left hand in water at 38 5 C.
5	656	7	19 84	16 71	4 37	3 70	Aneurysm first seven minutes
1	2,143	15	34 69	26 63	7 64	5 90	Last fifteen minutes in calorimeters
0	2,460	11	33 81	31 09	6 69	6 66	Aortic aneurysm
1	2,927	14	34 36	28 59	8 38	7 33	Thoracic aneurysm
9	2,974	14	44 50	38 82	10 23	9 47	
7	2,239	11	54 03	35 62	10 91	8 15	Innominate aneurysm
	517	4		23 89		5 47	Right hand in water at 8 1 C
	792	4		37 63		8 61	Right hand still in water at 8 1 C
.	413	3		26 91		6 16	Right hand in water at 4 3 C
	1,516	7		44 23		10 12	
6	4,292	13	64 47	62 28	11 11	10 32	Aortic aneurysm
7	208	14	2 07	2 73	0 41	0 57	Cardio-renal disease with aneurysm
4	100	8	2 81	2 43	0 17	0 14	Feet
5	501	17	5 65	5 36	1 11	1 18	
6	608	10	11 74	11 37	0 66	0 65	Feet
2	754	9	14 18	12 93	3 62	3 44	Raynaud's disease
4		12	20 18		5 16		Left hand in water at 43 C
0		3	9 23		2 46		Left hand in water at 12 C
5		4	15 36		3 93		Left hand still in water at 12 C
7		4	19 83		5 07		Left hand dried and wrapped
8	299	8	6 61	6 64	2 42	2 35	Raynaud's disease first eight minutes
5	1,079	16	8 83	12 12	3 23	4 29	Next sixteen minutes in calorimeters
2	415	7	9 97	10 73	3 65	3 80	Last seven minutes of the sixteen
0		2	17 89		6 55		Left hand in water at 43 C
1		10	11 74		4 30		Left hand still in water at 43 C
7		2	15 18		5 56		Left hand in water at 12 C
8		2	10 74		3 93		Left hand still in water at 12 C
7		5	1,752		6 41		Left hand still in water at 12 C
1	1,111	8	31 09	22 89	6 67	4 87	Arteritis in leg
	347	5		12 07		2 59	Right hand in water at 9 C
	589	4		26 06		5 59	Right hand still in water at 9 C
	382	3		22 97		4 93	Right hand in water at 43 C
	1,630	6		51 09		10 96	Right hand still in water at 43 C
4	336	10	4 96	5 16	1 22	1 12	Gangrene of fingers, septic endocarditis
3	1,199	15	11 59	13 75	2 65	3 32	Diabetes
	1,027	11		16 54		3 99	Right hand in water at 43 C
3	2,858	23	15 19	21 50	1 39	1 83	Feet (left gangrenous)
5	87	18	0 95	0 88	0 27	0 28	Myocarditis, cyanosis (Congenital heart?)
4		10	0 78		0 22		Left hand in water at 41 8 C
5	434	15	3 29	4 35	1 05	1 43	Cyanosis much less
2	4,712	23	20 47	33 77	2 15	3 42	Feet (left toes gangrenous)
	1,357	9		26 46		2 68	Right foot in water at 10 5 C
	1,726	11		28 30		2 87	Right foot in water at 42 C
7	300	R 11 L 9	6 32	4 90	2 16	1 64	Purpura
2		8	6 97		2 38		Left hand in water at 43 C
4		7	5 04		1 72		Left hand in water at 9 1 C
3	.	4	5 04		1 72		Left hand dried and wrapped
4	1,192	18	4 96	12 67	0 51	1 37	Feet (edema of right)
9	588	10	12 59	13 17	3 43	3 71	Hands
	210	4		11 83		3 33	Right hand in water at 42 8 C
	700	11		14 49		4 08	Right hand still in water at 42 8 C
	321	10		7 32		2 06	Right hand in water at 8 6 C

abrupt change in the sound occurred, the smaller number following is the pressure at which

CHRONIC GASTRIC ULCER AND ITS RELATION TO GASTRIC CARCINOMA*

REVIEW OF SIX HUNDRED EIGHTY-FOUR SPECIMENS †

WILLIAM CARPENTER MACCARTY, M D
AND
ALBERT COMPTON BRODERS, M D
MAYO CLINIC
ROCHESTER, MINN

The discussion which is going on in the literature over the question of whether or not gastric carcinoma develops on chronic gastric ulcer and regarding the percentage of chronic ulcers which "become" carcinomatous, is, it seems to us, a discussion which is not only dealing with an unanswerable question with our present methods of investigation, but is also doing a great deal of unnecessary harm to mankind by preventing rational treatment Viewed from the standpoint of feasibility of scientific investigation, such discussion seems superfluous for the following reasons 1 No one has seen a chronic gastric ulcer in the process of development 2 No one has been able to witness the stages of reaction to irritation through which the tissues of the gastric wall pass during the formation of ulcer or carcinoma 3 No one has ever recognized carcinoma in the process of development anywhere in man or other animal 4 Even the most skilful cellular pathologist cannot point out the line of demarcation between simple hyperplastic cells of the mucosa of a simple chronic ulcer and malignant hyperplastic cells of a chronic ulcer which is associated with carcinoma 5 No one has experimentally produced a carcinoma

With these fundamental limitations of our knowledge, one can only view the conditions as they occur The known facts which are seen in the specimens herewith presented are the following

1 Single and multiple chronic ulcerations occur in the stomach (Figs 1, 2, 13 and 18)

2 They vary widely in size (diameter and depth), as in Figures 1, 2, 5, 6, 8, 9, 10, 11, 13 14, 18, 19, 29 and 33

* Read before the Southern Minnesota Medical Society, Mankato, Aug 5, 1913, and before the Kentucky Medical Society, Sept 2, 1913

† This number includes 218 specimens previously reported by MacCarty at the meeting of the American Medical Association, June, 1909, and published in Surgery, Gynecology and Obstetrics, May, 1910

3 The characteristics of simple chronic gastric ulcers consist of excavations in the wall of the stomach, and these excavations may have the mucosa, muscularis mucosae, submucosa, musculature, subserosa or perigastric structures for their bases, depending on their depth (Figs 2, 3, 4, 6, 9, 11, 14, 19, 20, 29, 30, 34, 35, 37 and 38)

4 The excavations may be wide and shallow (Fig 2) or narrow and deep (Figs 8, 9, 37 and 38)

5 The mucosa of the borders of gastric ulcers may gradually recede from the base or overhang the base (Figs 2, 14 and 34)

6 The epithelium of the mucosa, which is the most natural source of carcinoma, may be normal (differentiated), as in Figures 15, 16 and 24, hyperplastic (partially differentiated), as in Figures 23 and 25, or irregularly hyperplastic and migratory (extra glandular or in the stroma), as in Figures 31, 32 and 36

7 The bases of chronic gastric ulcers consist of scar tissue which radiates usually from the center of the ulcer or is perpendicular to the plane of the coats of the stomach (Figs 7, 12, 20, 30, and 35)

8 Usually the surface of the base is composed of necrotic tissue which is infiltrated with lymphocytes (Figs 7, 12, 17, 20, 21, 30 and 35)

9 Carcinomatous cells occur in the lower parts of the mucosa and in the immediate submucosa without their being present in the base (Figs 30, 31 and 32)

10 Of 684 specimens which were either excised or resected from the stomach, 191 were chronic ulcers or ulcers in which no histologic evidence of carcinoma was present There were 472 specimens which presented the characteristics of simple ulcer plus the presence of carcinoma and twenty-one specimens of ulcer in which the presence of cancer was doubtful The ulcer which contains the smallest amounts of carcinomatous cells contains these in the mucosa of the borders and not in the base The earliest carcinomatous condition of the cells is not seen in so-called "cut-off" epithelium, in scar tissue of the base, but is seen in the epithelium of the mucosa (Figs 26, 27, 31 and 32)

Without regard to clinical history, certain conclusions may be drawn from the facts enumerated above

1 Single and multiple chronic ulcers occur in the stomach

2 Single and multiple chronic gastric ulcers occur with all the characteristics of simple ulcers plus the presence of carcinomatous cells in their borders minus the presence of similar cells in the bases

3 Simple and multiple gastric ulcers occur which present the macroscopic characteristics of simple ulcer plus the presence of carci-

noma in the borders and bases, indeed, with glandular involvement and metastases

The question for the clinician and the surgeon is not "Does gastric carcinoma develop on gastric ulcers and what percentage of ulcers become malignant?" but "Has the proved association of gastric carcinoma with chronic gastric ulcer sufficient significance to be of prognostic value in cases clinically diagnosticated as gastric ulcer?"

The association of these two conditions in conjunction with the fact that the immediate operative mortality, the postoperative mortality and the subsequent duration of life after gastric resection bear a definite relation to the extent of the carcinomatous involvement[1] should be sufficient to stimulate a consideration on the part of every physician of the possibility of chronic gastric ulcers not only becoming but actually being carcinomatous

The question of differential diagnosis in such cases may also be answered by facts the most salient of which is the fact that the differential diagnosis is often to be made only by a cellular pathologist after the removal of the tissue and not by the clinician or surgeon

Do we know of any method other than exploration and microscopic examination which positively answers the question of whether or not a specimen inside or outside the body is carcinoma? The clinical history, gastric analysis, roentgenoscopy and serum diagnosis cannot positively differentiate these groups of cases, especially those without pyloric obstruction Indeed, the microscope, which is our present highest court of appeal, can do no more than to divide the specimens into three groups, namely, simple ulcer group, carcinoma group and a doubtful group Whenever the clinician feels positive of the clinical diagnosis of chronic gastric ulcer he should consider that carcinoma can not be ruled by our present methods of clinical investigation

1 MacCarty and Blackford Involvement of Regional Lymphatic Glands in Carcinoma of the Stomach, Ann Surg, June, 1912



Fig 1 (18088) —Multiple chronic gastric ulcers in various degrees of extension

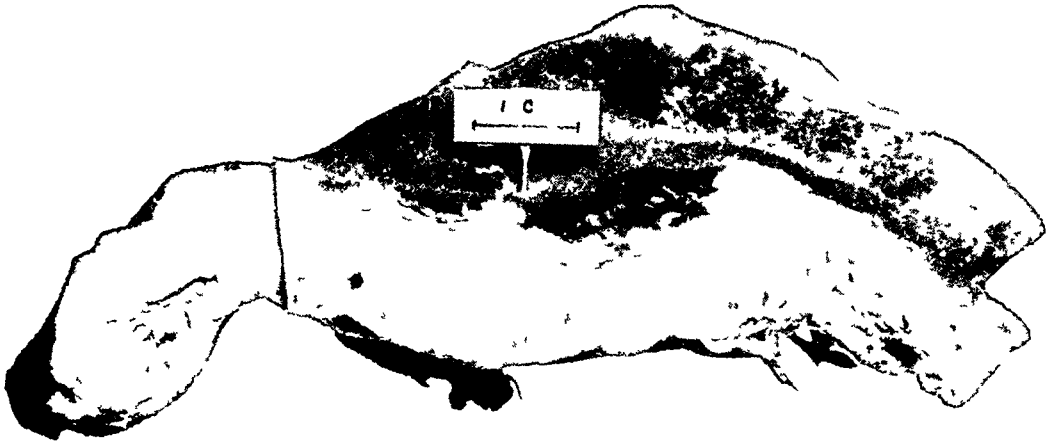


Fig 2 (18088) —Gross section through four ulcers showing the degrees of extension through the wall of the stomach



Fig 3 (18088) —Low-power photograph through the smallest ulceration in Figures 1 and 2 The destruction has not extended through the muscularis mucosae Some distortion of the glands and lymphocytic infiltration of the mucosa

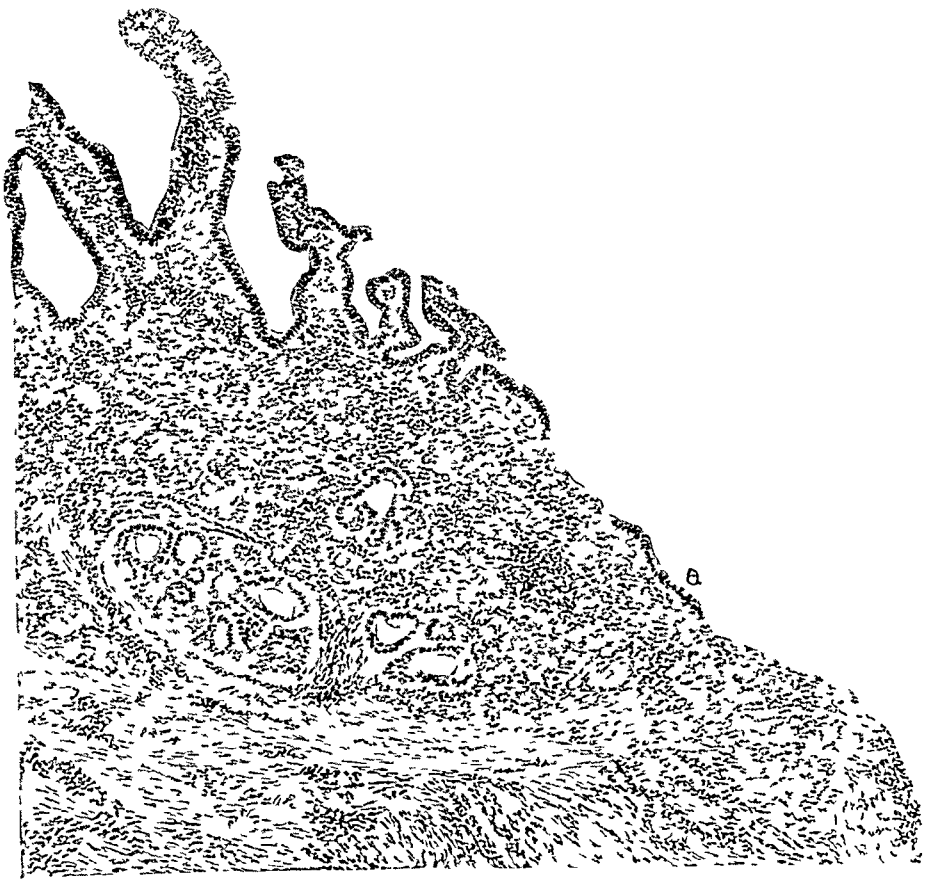


Fig 4 (18088) —Low-power drawing through the wall and part of the base of the ulcer second in size in Figures 1 and 2 The destruction has extended through the muscularis mucosae and into the submucosa There is extensive distortion of the glands and also lymphocytic infiltration



Fig 5

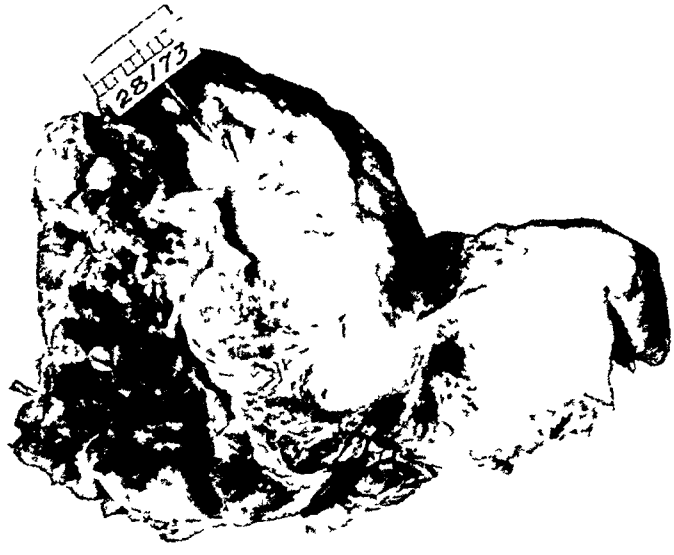


Fig 6

Fig 5 (28173) —Single simple chronic gastric ulcer

Fig 6 (28173) —Gross section through the ulcer in Figure 5 showing the depth and extensive scar tissue of the base The ulceration has extended through all of the gastric coats



Fig 7 (28173) —Low-power drawing through the specimen in Figures 5 and 6 showing the characteristics of a simple chronic gastric ulcer Destruction, distortion of the mucosa, lymphocytic infiltration, necrosis of the tissue in the base, and the peculiar arrangement of the scar tissue in the base

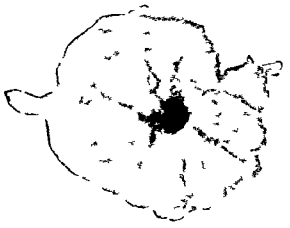


Fig 8



Fig 9



Fig 10



Fig 11

Figs 8, 9, 10 and 11 (37378 and 40640) —Photographs showing simple chronic gastric ulcers

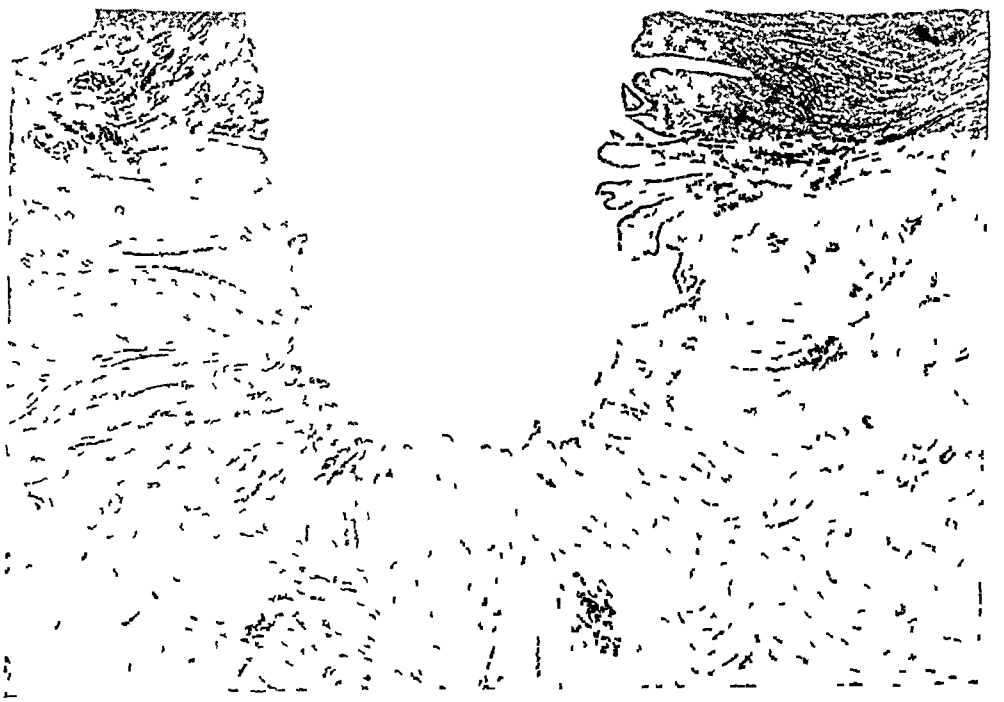


Fig 12 (27381) —Low power drawing showing the characteristics of a simple chronic gastric ulcer



Fig 13



Fig 14

Figs 13 and 14 (49687) —Photographs of a simple chronic gastric ulcer



Fig 15

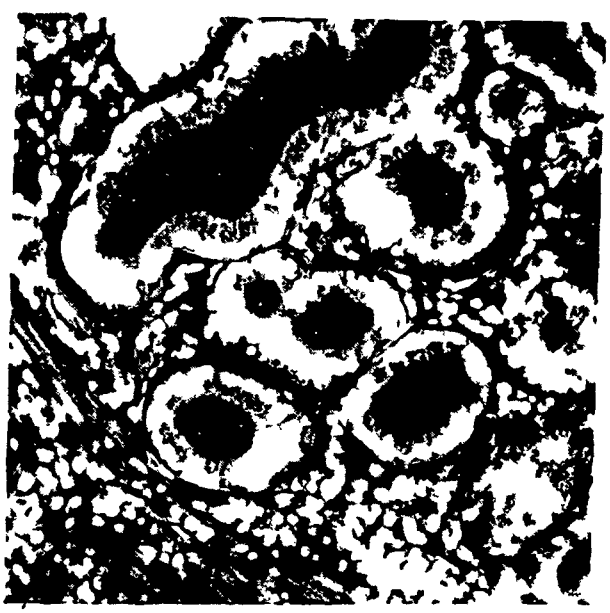


Fig 16

Figs 15 and 16 (48401) —Low power photographs showing the character of the glands of the mucosa in a simple chronic gastric ulcer The epithelium consists of definitely differentiated cells which still retain the characteristics of the normal gastric epithelium



Fig 17 (48401) —Low-power photograph through the base of a simple chronic gastric ulcer showing lymphocytic infiltration and scar tissue



Fig 18



Fig 19

Figs 18 and 19 (A53435) —Photographs of a simple chronic gastric ulcer

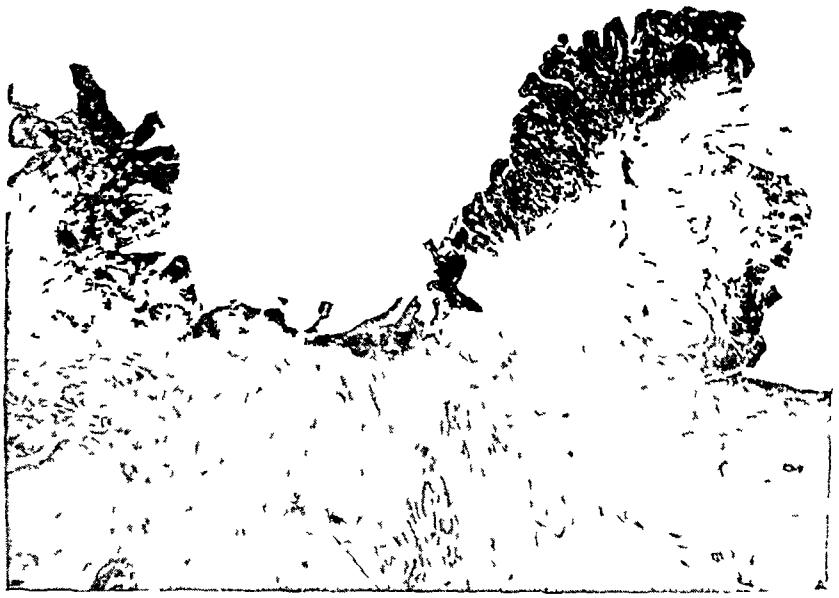


Fig 20 (53630) —Low power photograph through a simple chronic gastric ulcer



Fig 21 (53630) —Low-power photograph through the base of the ulcer in Figure 21



Fig 22



Fig 23



Fig 24



Fig 25



Fig 26

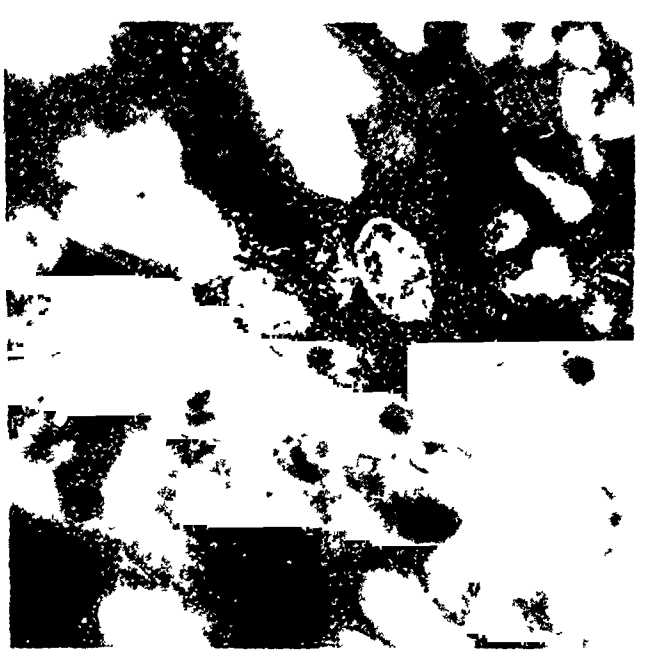


Fig 27

Figs 22 23 24, 25, 26 and 27 (53630) —Photograph of the epithelium in the mucosa of the borders of a chronic gastric ulcer (Fig 20) The glands are more irregular than those seen in Figures 15 and 16 The cells are not so typically differentiated as those in Figure 16, and still there is no definite invasion of the stroma The cells resemble many of those which are seen in carcinoma (Figs 31 32 and 36)

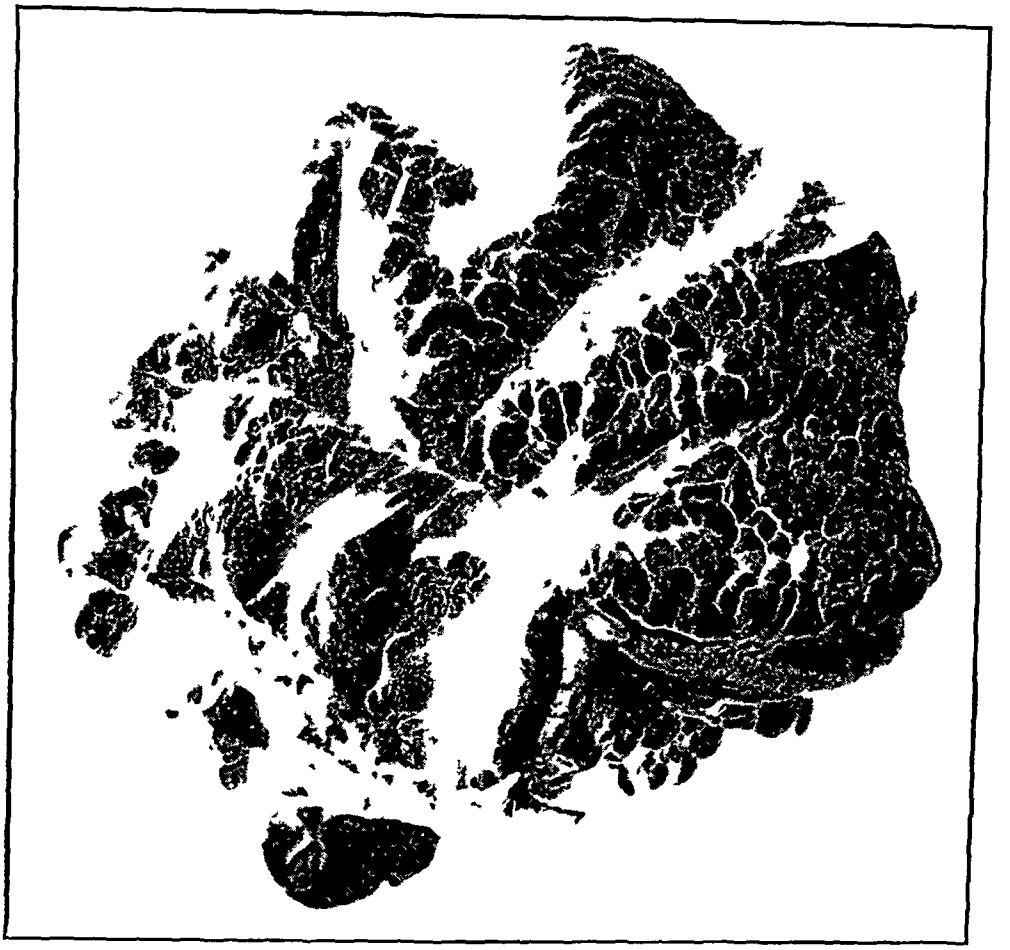


Fig 28



Fig 29

Figs 28 and 29 (35572) —Photographs through a chronic gastric ulcer



Fig 30 (35572) —Low-power photograph through the ulcer which is seen in Figures 28 and 29 The characteristics of simple chronic gastric ulcer may be seen to exist There is no apparent carcinoma in the base



Fig 31



Fig 32

Figs 31 and 32 (35572) —*Photographs of undifferentiated, irregular epithelial* cells in the submucosa just below the muscularis mucosae The cells are typical of carcinoma



Fig 33

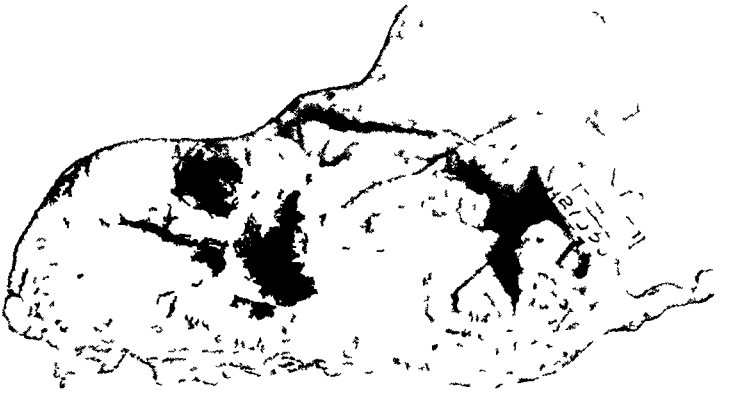


Fig 34

Figs 33 and 34 (21555) —Photographs of chronic gastric ulcers



Fig 35 (21555) —Low power drawing through the border and part of the base of the ulcer, showing the presence of carcinoma in the mucosa and just below the mucosa, but none in the base Direct connection between the glands of the mucosa and the carcinoma may be seen



Fig 36



Fig 37

Fig 36 (21555) —High-power drawing through an epithelial carcinomatous island in the submucosa of the gastric ulcer in Figs 33 and 34

Fig 37 (21882) —Chronic gastric ulcer plus the presence of carcinoma

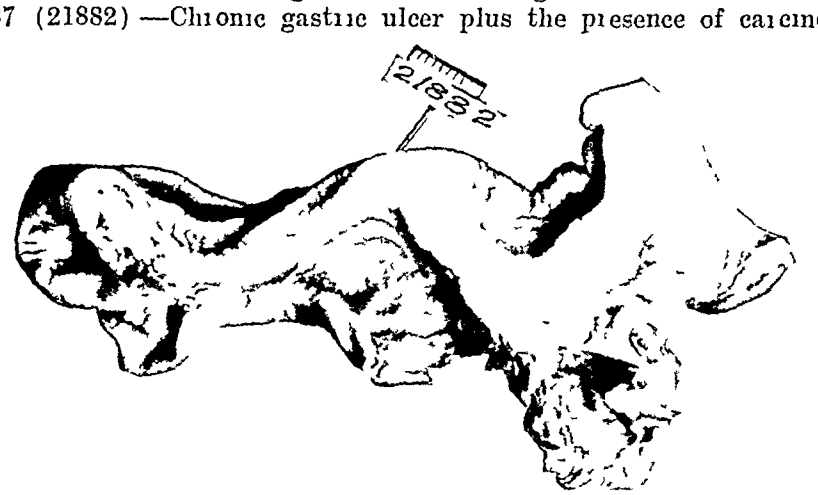


Fig 38 (21882) —Gross section through the carcinomatous ulcer which is seen in Figure 37

THE NON-PROTEIN NITROGENOUS CONSTITUENTS OF THE BLOOD IN CHRONIC VASCULAR NEPHRITIS (ARTERIOSCLEROSIS) AS INFLUENCED BY THE LEVEL OF PROTEIN METABOLISM *

OTTO FOLIN, PH D, W DENIS, PH D, AND MALCOLM SEYMOUR, M D
BOSTON

By virtue of the analytical technic recently introduced by Folin and Denis for the analysis of blood it has become possible to study the retention of nitrogenous waste products with far greater ease and certainty than formerly Such studies are particularly to be desired in the investigation of nephritis, for urine analyses cannot reveal the stage of accumulation of such products, an accumulation which may vary between almost normal figures and those accompanying uremia According to Strauss, the high blood-pressure of nephritis is usually or at least frequently accompanied by an excessive accumulation of the nitrogenous waste products, whereas edema is rather associated with a retention of chlorids For the investigation reported in this paper we have selected a group of patients whose blood-pressure varied between 160 and 225 mm

The chief purpose of our investigation was to determine the extent to which it is possible by means of diets to vary, particularly to reduce, the waste nitrogen in the blood of such nephritis and incidentally determine whether there is any relationship between the blood-pressure and the accumulations of nitrogenous waste products in the blood With regard to the latter point, the details of our results will be described by one of us (Seymour) in a separate paper, though a few figures for the blood-pressure are recorded in Tables 1 to 12 We have included in the work a series of "kidney efficiency" tests, according to the phenolsulphonephthalein method recently introduced by Rowntree and Geraghty, in order to obtain further comparisons between the values given by that test and the actual accumulation as revealed by blood analyses While hospital records were kept of the diet of each patient, we omit all such data and give the urinary nitrogen alone as the index of the total protein metabolism The subjects of these experiments were inmates of an institution maintained on Long Island, Boston Harbor, by the city of Boston for the accommodation of indigent individuals They were

* From the Laboratories of Biologic Chemistry and Theory and Practice of Harvard Medical School and the Long Island Hospital

* Submitted for publication Oct 6, 1913

elected mainly on the basis of abnormally high blood-pressures, and vere removed from the institute and placed in a special ward in the Long Island Hospital.

With the exception of Charles M, all of these patients were allowed o be out of bed during the day, but in order to control the collection of ine and the diets, they were not permitted to leave their own ward, xcept to go on an adjoining veranda The patients were under the constant supervision of a special nurse and orderly, who were placed at our disposal for this purpose by the hospital authorities

It was originally our intention to divide the time covered by the xperiment into three periods (1) a period in which the patients would be allowed to eat at will the ordinary ward diet, (2) a period of high protein feeding, (3) a period of low protein diet with high calorific ntake The diets proposed for the first and third periods were successfully administered, but during the period of high protein feeding we vere, as will be seen on examination of the figures for urinary nitrogen, unable to raise the protein metabolism to a very high level This failure vas due to the nausea and vomiting which occurred in many cases during hat part of the experiments

During the second or "high protein" period beside the administration of a liberal supply of meat and eggs at the three regular meals, two to hree egg-nogs daily were given between meals

During the third or low protein period the diet consisted of apple-apioca pudding, boiled or fried rice, boiled potatoes with butter, fruit (oranges, apples and stewed pears) and boiled cabbage In addition, during this period, each patient took daily 150 to 200 gm of arrowroot starch, which had been rendered soluble with diastase This soluble starch was served hot, flavored with fruit juice or with a little port wine or whisky

During the entire experiment smoking was allowed, and coffee was served three times daily

The crucial point which we set out to determine, namely, whether by low nitrogen diets it is possible to reduce the urea and total non-protein nitrogen of blood to the normal or less is abundantly proven by the tabular records of the preceding twelve nephritic patients In every case the figures for the retention nitrogen obtained at the end of the third period, the low protein period, are down substantially to the figures obtained from strictly normal persons (from 22 to 28 mg non-protein nitrogen and from 11 to 14 mg urea nitrogen)

The original level of retention was, to be sure, not very high in several of these patients The patients had been selected chiefly on the basis of the blood-pressure, and high blood-pressure is not necessarily accompanied by excessive retention of nitrogenous waste products Judging

TABLE 1.—RESULT OF TEST IN CASE 1

Daniel B., aged 60, weight 63.5 kg. Diagnosis: Aortic roughening and regurgitation, chronic interstitial nephritis.

Diet	Date	Urine		Blood (100 gm.)			Phenol sulphonephthalein Excretion in 2 Hrs. Per Cent	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	960	9.7				26	175
Ward	4/11/13	1,080	9.9					
Ward	4/12/13	930	9.7					
Ward	4/13/13	1,020	9.2					
Ward	4/14/13	1,050	8.4	40	18	1.9	22	145
High protein	4/16/13	1,020	8.9					160
High protein	4/17/13	1,080	10.3					
High protein	4/18/13	1,140	12.6				38	
High protein	4/20/13	1,560	11.2					
High protein	4/21/13	1,020	9.7	40	20	2.7	26	155
Low protein	4/23/13	1,020	7.9					140
Low protein	4/24/13	1,050	11.5					
Low protein	4/25/13	1,080	5.5					
Low protein	4/26/13	1,080	3.5					
Low protein	4/27/13	1,200	2.7	21	11	2.5	45	170
Average							31.6	

TABLE 2.—RESULT OF TEST IN CASE 2

Thos. H. M., aged 57, weight 80.5 kg. Diagnosis: Chronic interstitial nephritis, hypertrophy of heart, arteriosclerosis.

Diet	Date	Urine		Blood (100 gm.)			Phenol-sulphonephthalein Excretion in 2 Hrs. Per Cent	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	960	8.4					180
Ward	4/11/13	450	5.1					
Ward	4/12/13	780	7.4					
Ward	4/13/13	1,080	8.4					
Ward	4/14/13	1,140	8.0	44	26	1.7	12	190
High protein	4/16/13	900	7.5					185
High protein	4/17/13	1,020	8.1					
High protein	4/18/13	1,140	8.4				22	
High protein	4/20/13	1,620	12.4					
High protein	4/21/13	1,560	15.0	44	24	2.7	36	160
Low protein	4/23/13	1,440	9.8					190
Low protein	4/24/13	900	6.6					
Low protein	4/25/13	1,500	6.0					
Low protein	4/26/13	1,200	4.4					
Low protein	4/27/13	1,630	4.2	19	10	1.7	35	208
Average							26.2	

TABLE 3.—RESULT OF TEST IN CASE 3

William McG., aged 71, weight 67 5 kg. Diagnosis: Arteriosclerosis, chronic emphysema, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	900	7 0					160
Ward	4/11/13	510	5 1					
Ward	4/12/13	900	9 0					
Ward	4/13/13	1,020	7 5					
Ward	4/14/13	1,350	7 8	38	20	2 2	34	160
High protein	4/16/13	1,020	9 2					160
High protein	4/17/13	905	8 2					
High protein	4/18/13	1,020	10 8				29	
High protein	4/20/13	1,380	11 3					
High protein	4/21/13	1,080	10 6	37	20	2 3	34	160
Low protein	4/23/13	1,920	7 4					145
Low protein	4/24/13	900	9 0					
Low protein	4/25/13	1,440	5 3					
Low protein	4/26/13	1,500	7 1					
Low protein	4/27/13	1,260	3 3	23	11	2 0	37	158
Average							33 5	

TABLE 4.—RESULT OF TEST IN CASE 4

Timothy, W. C., aged 71, weight 91 5 kg. Diagnosis: Right inguinal hernia, arteriosclerosis, senility, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Nitrogen Protein mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13							175
Ward	4/11/13	570	5 4					
Ward	4/12/13	1,560	11 2					
Ward	4/13/13	1,230	8 8					
Ward	4/14/13	2,070	12 0	49	25	2 6		170
High protein	4/16/13	1,560	10 1					142
High protein	4/17/13	1,440	13 7					
High protein	4/18/13	1,560	14 8				31	
High protein	4/20/13	1,080	16 2					
High protein	4/21/13	1,440	18 7	48	23	2 6	39	165
Low protein	4/23/13	1,080	8 7					140
Low protein	4/24/13	1,050	10 4					
Low protein	4/25/13	1,620	6 5					
Low protein	4/26/13	1,980	5 9					
Low protein	4/27/13	2,040	5 2	22	10	1 8	50	158
Average							46 6	

TABLE 5.—Result of Test in Case 5

Nathan, D L, aged 81, weight 78 5 kg Diagnosis Arteriosclerosis, senility, cardiac hypertrophy, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm)			Phenol-sulphone-phthalein Excretion in 2 Hrs Per Cent	Blood-Pressure
		Vol c c	Nitrogen gm	Non Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg		
Ward	4/ 9/13	1,290	7 8					210
Ward	4/11/13	600	5 7					
Ward	4/12/13	1,110	7 8					
Ward	4/13/13	1,140	8 5					
Ward	4/14/13	1,590	12 2	50	28	2 7	14	198
High protein	4/16/13	1,260	8 4					188
High protein	4/17/13	990	8 5					
High protein	4/18/13	1,200	12 0				20	
High protein	4/20/13	1,440	11 0					
High protein	4/21/13	1,050	10 0	58	40	3 3	17	235
Low protein	4/23/13	1,440	6 7					170
Low protein	4/24/13	1,080	6 9					
Low protein	4/25/13	960	4 5					
Low protein	4/26/13	1,500	5 7					
Low protein	4/27/13	1,920	4 3	23	12	2 0	28	203
Average							19 8	

TABLE 6.—Result of Test in Case 6

Martin, T D, aged 54, weight 71 5 kg Diagnosis Arteriosclerosis, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm)			Phenol-sulphone-phthalein Excretion in 2 Hrs Per Cent	Pressure Blood-
		Vol c c	Nitrogen gm	Non-Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg		
Ward	4/ 9/13	1,260	11 1				35	235
Ward	4/11/13	1,050	10 5					
Ward	4/12/13	930	6 4					
Ward	4/13/13	1,530	7 8					
Ward	4/14/13	1,710	8 7	39	18	3 0	33	187
High protein	4/16/13	1,020	8 3					200
High protein	4/17/13	1,500	13 1					
High protein	4/18/13	1,260	9 6				23	
High protein	4/20/13	2,040	14 1					
High protein	4/21/13	1,620	13 6	62	45	3 9	30	180
Low protein	4/23/13	1,320	8 5					160
Low protein	4/24/13	1,200	7 7					
Low protein	4/25/13	1,500	6 1					
Low protein	4/26/13	1,560	5 6					
Low protein	4/27/13	1,560	4 8	30	15	2 7	29	190
Average							30	

TABLE 7.—Result of Test in Case 7

Edward R., aged 74, weight 59.5 kg. Diagnosis: Inguinal hernia, arteriosclerosis, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent	Blood-Pressure
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	1,260	7.4					225
Ward	4/11/13	840	6.8					
Ward	4/12/13	1,410	9.4					
Ward	4/13/13	1,350	10.3					
Ward	4/14/13	1,250	9.8	50	28	2.2	21	190
High protein	4/16/13	1,380	9.1					200
High protein	4/17/13	1,140	9.5					
High protein	4/18/13	1,860	11.5				29	
High protein	4/20/13	1,920	11.7					
High protein	4/21/13	1,380	11.1	82	67	3.9	26	180
Low protein	4/23/13	1,500	6.7					190
Low protein	4/24/13	1,440	7.5					
Low protein	4/25/13	1,560	4.9					
Low protein	4/26/13	1,080	3.3					
Low protein	4/27/13	900	1.9	26	12	2.3	35	190
Average							28	

TABLE 8.—Result of Test in Case 8

Michael O'C., aged 66, weight 90.5 kg. Diagnosis: Arteriosclerosis, chronic emphysema, cardiac hypertrophy, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm.)			Phenolsulphonephthalein Excretion in 2 Hrs. Per Cent	Pressure Blood-
		Vol. c.c.	Nitrogen gm.	Non-Protein Nitrogen mg.	Urea Nitrogen mg.	Uric Acid mg.		
Ward	4/ 9/13	1,110	10.1					195
Ward	4/11/13	720	6.7					
Ward	4/12/13	1,050	11.4					
Ward	4/13/13	1,260	9.9					
Ward	4/14/13	1,170	11.1	28	14	2.5	34	198
High protein	4/16/13	1,440	11.1					240
High protein	4/17/13	1,380	11.3					
High protein	4/18/13	1,020	10.6				23	
High protein	4/20/13	1,446	14.4					
High protein	4/21/13	1,680	15.3	30	15	2.5		210
Low protein	4/23/13	1,080	10.8					180
Low protein	4/24/13	1,320	10.9					
Low protein	4/25/13	1,440	6.3					
Low protein	4/26/13	1,020	2.4					
Low protein	4/27/13	1,500	3.8	20	11	2.6	30	150
Average							29	

TABLE 9.—Result of Test in Case 9

William T W, aged 59, weight 70 5 kg Diagnosis Left hemiplegia, mitral regurgitation and aortic roughening, chronic interstitial nephritis, arteriosclerosis

Diet	Date	Urine		Blood (100 gm)			Phenolsulphonephthalein Excretion in 2 Hrs Per Cent	Blood-Pressure
		Vol c c	Nitrogen gm	Non-Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg		
Ward	4/ 9/13	720	4 8					175
Ward	4/11/13	480	5 1					
Ward	4/12/13	1,050	5 4					
Ward	4/13/13	1 020	9 8					
Ward	4/14/13	1,110	7 4	27	14	2 6		170
High protein	4/16/13	1,020	7 0					172
High protein	4/17/13	1,080	9 6					
High protein	4/18/13	1,080	9 0				26	
High protein	4/20/13	1,560	13 0					
High protein	4/21/13			30	16	2 8	31	140
Low protein	4/23/13	1,500	7 0					160
Low protein	4/24/13	1,050	7 8					
Low protein	4/25/13	1 200	3 5					
Low protein	4/26/13	720	2 6					
Low protein	4/27/13	1 620	3 3	20	11	2 3	24	190
Average							27	

TABLE 10.—Result of Test in Case 10

John De W, aged 82, weight 80 kg Diagnosis Cardiac hypertrophy, arteriosclerosis, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm)			Phenolsulphonephthalein Excretion in 2 Hrs Per Cent	Blood-Pressure
		Vol c c	Nitrogen gm	Non-Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg		
Ward	4/ 9/13	750	7 3				23	170
Ward	4/11/13	540	5 8					
Ward	4/12/13	600	6 4					
Ward	4/13/13	1,110	9 2					
Ward	4/14/13	1,470	7 9	30	15	2 5	35	150
High protein	4/16/13	1,020	8 2					154
High protein	4/17/13	1,200	12 0					
High protein	4/18/13	960	12 5				16	
High protein	4/20/13	2,040	10 1					
High protein	4/21/13	1,500	19 6	30	15	3 0	18	145
Low protein	4/23/13	1,020	7 7					135
Low protein	4/24/13	1,050	9 8					
Low protein	4/25/13	1 440	5 3					
Low protein	4/26/13	1,560	4 7					
Low protein	4/27/13	1 560	3 4	19	10		20	140
Average							28	

TABLE 11—RESULT OF TEST IN CASE 11

Frank M, aged 72, weight 89 5 kg Diagnosis Arteriosclerosis, cardiac hypertrophy, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm)			Phenol-sulphone-phthalein Excretion in 2 Hrs Per Cent	Blood-Pressure
		Vol c c	Nitrogen gm	Non-Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg		
Ward	4/ 9/13	1,110	15 0					225
Ward	4/11/13	570	5 6					
Ward	4/12/13	1,050	8 5					
Ward	4/13/13	1,560	9 5					
Ward	4/14/13	2,040	9 6	30	15	2 6	29	190
High protein	4/16/13	1,440	11 5					220
High protein	4/17/13	1,560	12 3					
High protein	4/18/13	1,560	14 2				27	
High protein	4/20/13	1,560	13 0					
High protein	4/21/13	1,920	14 8	32	16	2 8	39	200
Low protein	4/23/13	1,020	7 0					180
Low protein	4/24/13	1,440	7 5					
Low protein	4/25/13	1,680	4 8					
Low protein	4/26/13	1,620	4 6					
Low protein	4/27/13	2,100	4 2	19	10	2 0	33	200
Average							32	

TABLE 12—RESULT OF TEST IN CASE 12

Chas McL, aged 63, weight 90 5 kg Diagnosis Cardiac hypertrophy, arteriosclerosis, chronic interstitial nephritis

Diet	Date	Urine		Blood (100 gm)			Phenol-sulphone-phthalein Excretion in 2 Hrs Per Cent	Pressure Blood-
		c c Vol	Nitrogen gm	Non-Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg		
Ward	4/ 9/13	1,740	13 9					150
Ward	4/11/13	960	10 5					
Ward	4/12/13	1,020	11 1					
Ward	4/13/13	1,200	9 3					
Ward	4/14/13	1,530	9 5	30	14	3 0	13	160
High protein	4/16/13	1,440	9 9					139
High protein	4/17/13	1,560	12 2					
High protein	4/18/13	1,500	15 5				45	
High protein	4/20/13	1,920	18 3	37	20	2 9	33	140
Low protein	4/23/13	2,400	12 5					
Low protein	4/24/13	1,380	8 4					
Low protein	4/25/13	2,400	6 2					
Low protein	4/26/13	2,400	4 6					
Low protein	4/27/13	1,560	3 4	20	11	2 1	28	140
Average							29 7	

from our figures, there clearly is no marked connection between the blood-pressure and the degree of retention, indeed, it is doubtful whether there is any connection A high blood-pressure, therefore, does not necessarily indicate the desirability of introducing low nitrogen diets in nephritis So far as can be seen at the present time, the only criterion available for regulating the protein content of the food in nephritis is the determination of the amount of retention by blood analysis Five of the twelve patients (the last five) used in our experiments showed little or no abnormal retention of waste nitrogen, and there is no reason to doubt that these could take care of the protein contained in the usual hospital diet

So far as the phenolsulphonephthalein test for the kidney efficiency is concerned, it is to be noted that there is no correspondence between the results of this test and the degree of waste nitrogen retention Since it is possible to increase or reduce the retention by protein feeding one cannot expect any close agreement between it and the phenolsulphonephthalein test The lack of correspondence is, however, probably not to be explained entirely on the basis of different levels of total protein metabolism

In Table 13 we give the nitrogen elimination, the non-protein nitrogen and the phenolsulphonephthalein excretion — all in terms of the average for the first two periods of the experiments

TABLE 13 —AVERAGE IN CASES 1 TO 12 OF URINARY NITROGEN, NON PROTEIN NITROGEN AND PHENOLSULPHONEPHTHALEIN EXCRETION*

Case	Urine Nitrogen gm	Blood Non-Protein Nitrogen, mg	Phenolsulphonephthalein Excretion Per Cent
9	8 0	28 5	27 0
3	8 7	37 5	33 5
2	8 9	44 0	26 2
5	9 2	54 0	19 8
7	9 7	61 0	28 0
1	9 8	40 0	31 6
6	10 3	50 5	30 0
10	10 9	30 0	28 0
8	11 0	29 0	29 0
11	11 4	31 0	29 5
12	12 3	33 5	29 7
4	14 3	48 5	46 6

* Average for first two periods arranged according to urinary nitrogen

If 60 to 80 per cent be taken as the normal elimination of 6 mg of phenolsulphonephthalein excretion when injected intramuscularly it is clear that this elimination can be reduced by one-half or more before there need be any abnormal accumulation of waste nitrogen in the blood

TABLE 14.—Result of Blood Tests in Cases 13 to 18

Case	Name	Age	Diagnosis	Blood									Phenol-sulphone-phthalein Excretion Per Cent in 2 Hrs
				Ward Diet			High Protein Diet			Low Protein Diet			
				Non-Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg	Non-Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg	Non-Protein Nitrogen mg	Urea Nitrogen mg	Uric Acid mg	
13	*Patrick M	63	Chr interstitial neph	92	70	3 5	124	109	4 5				8
14	Mary K O	75	Chr interstitial neph	42	34	2 5	61	41	3 5				54
15	Hannah J	64	Chr interstitial neph, aortic roughening, aneurysm of arch	50	34	2 1	58	40	2 5	36	16	2 4	32
16	Mary K	81	Chr interstitial neph cardiac hypertrophy, aortic roughening, mitral regurgitation	35	19	1 9	39	25	1 9	30	16	2 0	30
17	†Chas M	60	Chr interstitial neph, epilepsy	34	19	2 6	69	55	2 0	23	11		25
18	Dennis O'B	31	Chr interstitial neph albuminuric retinitis, alcoholic	58	41	2 0	‡75 80	52 62	2 1 2 5	44	20	2 1	6

* This patient died in "uremia" while on high protein diet two days after last sample of blood was taken

† This patient was confined to bed during almost the whole period of experiment Urinary nitrogen determinations made March 4 to April 4 showed nitrogen excretion varied from 22 to 15 gm in twenty-four hours Went on low protein diet, as given in first twelve cases, April 23 During a five day period nitrogen excretion average in twenty-four hours was 5 gm

‡ Taken one week apart

It would seem from these results as though the direct determination of the non-protein nitrogen (and urea) in the blood furnishes a more reliable guide to what might be called the protein tolerance of patients than can be obtained from any "direct" test of kidney efficiency, for of all tests yet devised for this purpose the phenolsulphonephthalein test of Rowntree and Geraghty is admittedly the best

In addition to the preceding experiments we have made a number of others which are less complete, but from which some noteworthy observations were obtained These experiments were made before the series shown in Tables 1 to 13, and while in every case collections of urine were made it was suspected that in a number of instances the whole twenty-four-hour amount had not been saved We, therefore, have not included our determinations of urinary nitrogen, but have been forced to rely on the dietary records kept by the nurses in charge

As in the preceding experiments, eggs and meat were used in liberal amounts during the periods of high protein feeding In the low protein periods, however, the patients were fed on cereals, bread and butter, vegetables and a little milk The soluble starch employed so extensively in our later experiments was not used, so that in some cases it is to be feared that the calorific intake was too low

UROBILIN ITS CLINICAL SIGNIFICANCE*

RAY LYMAN WILBUR, M D, AND THOMAS ADDIS, M D
SAN FRANCISCO

I INTRODUCTION

The numerous studies made on urobilin and urobilinogen during the forty-four years since its discovery by Jaffé, while adding much to our knowledge of its chemistry and its clinical occurrence, still leave much to be desired as to methods for its estimation and as to its clinical significance Some investigators are inclined to look on it still from the point of view of hemoglobin metabolism, while others consider it only in reference to hepatic insufficiency Its relationship to the presence or absence of bile in the intestine has been a subject of considerable dispute, but in the main the contention of Friedrich von Muller, that it can originate only from the bacterial decomposition of bile reaching the intestine, is accepted Nevertheless, the assertion of Fischler, that it can originate in the liver itself, seems to us probable Whether it is then formed within the liver cell or is produced by decomposition of bilirubin in the biliary passages, it is impossible to state. A wide variability in point of view is reflected throughout the available literature It has been our intention in entering on a study of this substance to endeavor to estimate its present value as an aid in diagnosis and prognosis, and we must frankly admit that it has been disappointing in some particulars, owing largely, perhaps, to the imperfections of the present methods of its estimation, and to its evident instability Besides, when such variable factors as the bacterial fermentations within the intestine, the absorption from it, the activity of the liver, the elimination of bile, the eliminative power of the kidney, the rate of destruction of the red blood-corpuscles, the activity of the spleen, etc, have to be taken into account, it is not surprising that difficulty should arise in interpreting findings and in correlating the diverse views of many investigators and clinicians

The interesting spectroscopic fluorescent and color tests for urobilin and its mother substance urobilinogen and their common occurrence in the body excretions, especially in disease, naturally stimulated much research as to their composition and origin and many clinical observations as to their significance The Ehrlich aldehyd test, shown by

* Submitted for publication July 1, 1913

* From the Laboratory of Experimental Medicine, Leland Stanford Junior University, Department of Medicine

Neubauer to be due to urobilinogen, received particular attention The method of estimation with Citron's or other simple spectroscope of the urobilin and urobilinogen together proved with us to be fairly simple and to lend itself readily to approximate quantitative studies based on the disappearance of the characteristic absorption bands between D and E, and B and F, with successive dilutions

The term "urobilin" is not applied to a single body, but to a group of derivatives of blood and bile pigments containing the pyrrol nucleus Throughout our work we have considered urobilin and its mother substance urobilinogen as practically the same, and have endeavored always to estimate them together in making clinical and experimental observations The confusion between the two and the lack of a generally accepted theory of the origin of these substances is the cause of the present striking lack of uniformity in the interpretation of clinical and laboratory results

The most prominent of the various theories as to the origin of urobilin are the hematogenous, the hepatogenous, the nephrogenous, the histogenic and the enterogenous The latter is undoubtedly the most satisfactory, although it does not fully explain the origin of all urobilin within the body, for instance, that of some hepatic disorders (Fischler) and some cases of blood destruction and extravasation In brief these theories are as follows

Hematogenous Theory.—This theory is that the urobilin can be but is not necessarily, derived directly from blood pigment It is based on the somewhat uncertain evidence of the long-known occurrence of urobilinuria in hemorrhage of the brain (von Bergmann), in blood extravasations (Kunkel), in extra-uterine pregnancy (Dick), in hemorrhagic ascites (such as the famous case of Gerhardt, in which there was also a carcinomatous closure of the ductus choledochus), after hemocytolytic processes, in sulphonal poisoning, in paroxysmal hemoglobinuria and in scurvy While it is true that there is the possibility in many of these reported clinical observations of the existence of definite hepatic disturbance, still there is enough experimental evidence to warrant the assertion that at times urobilin can be derived directly from blood pigment without the intervention of either the liver or the intestine

Hepatogenous Theory.—The principal tenet of this unsatisfactory theory is that there is failure on the part of the liver in its normal decomposition of hemoglobin, and that as a result of this insufficiency urobilin is formed and reaches the kidney through the blood-stream and is there eliminated, or is added to the intestinal contents by the bile A somewhat strict interpretation of this theory is that there is a direct formation of urobilin within the liver cell, or at least within the bile passages from the bile pigment

Nephrogenous Theory.—In brief, this is based on the fact that urobilin as such is very rarely found in the blood, and that it reaches the kidney in the form of bilirubin, which is reduced to urobilinogen in its passage through the renal epithelium Herscher states that kidney substance can reduce bilirubin, but surviving kidneys with bilirubin circulating in their blood secrete no urobilinogen, but only bilirubin in the collected urine The principal argument against this theory is that urobilin does not occur in many cases of jaundice

Histogenic Theory—This theory that hemoglobin or bile pigments are reduced in the tissues to urobilin and then reabsorbed and eliminated, offers an insufficient explanation of the various known phenomena

Enterogenous Theory (Friedrich von Muller)—While not meeting all conditions, this theory is based on the explanation of the established facts of urobilin formation In brief, it is based on the finding that bile pigment reaching the intestinal lumen undergoes a change into urobilinogen and urobilin caused by bacterial decomposition It may then be absorbed into the capillaries of the portal system and so reach the liver parenchyma If the liver is normal the major portion of the urobilin is broken up or synthesized to bile or blood pigment A portion may be secreted in the bile and so be returned to the intestinal lumen to be reabsorbed or passed off with the stool When the liver parenchyma is damaged, these changes in the urobilin may not take place, but it passes on into the general circulation It reaches the kidney through the blood and is eliminated in the urine This theory is principally sustained by the absence of urobilin in the urine in any quantity in cases of obstruction of the common duct

We shall not stop for a complete discussion of these theories, since points of view vary, and there is no common agreement at present We can safely assume that as a rule the presence of urobilinuria is indicative of the fact that bile is reaching the intestine, and that large amounts of urobilin in the urine usually mean that there has been either abnormal destruction of red blood-corpuscles, or an insufficiency of the liver to perform its normal functions A large amount of urobilin in the stool indicates increased blood destruction Urobilinuria frequently disappears in severe diarrhea Although urobilin is normally present in the urine in small quantities, its presence cannot be ascertained by the ordinary qualitative tests — a positive reaction with Schlesinger's zinc acetate test or Ehrlich's aldehyd test is not found in normal urine It is increased in many pathological conditions It is commonly increased in infectious diseases, in diseases in which there is wide-spread hemorrhage, traumatic or otherwise, in pernicious anemia, malaria, cardiac decompensation, various forms of liver and gall-passage disease, and in numerous intoxications such as those due to alcohol, chloroform, lead and carbon monoxid

II SUMMARY OF QUANTITATIVE METHODS FOR UROBILIN ESTIMATION

Following is a summary of only the most essential points of the methods which have been used

Almost all the work done has been with the urine The methods may be divided roughly into those requiring a separation of the urobilin from the urine by means of precipitation with ammonium sulphate, secondly, those depending on the spectroscopic absorption bands of urobilin, and thirdly, those based on the property of fluorescence and lastly colorimetric methods depending on the violet color produced by copper sulphate in urobilin solutions

1 Methods Involving Salting out of Urobilin by Means of Ammonium Sulphate—Hoppe Seyler in 1891 estimated urobilin in urine gravimetrically The acidified urine was saturated with ammonium sulphate, and the precipitate extracted with chloroform and alcohol The solution was evaporated, the residue dissolved in ether, filtered and evaporated, and the residue dissolved in alcohol, evaporated and weighed

Viglezio in the same year recommended a method which depended on the salting out of the urobilin with ammonium sulphate and its solution in alcohol The amount was gaged by noting how much of this solution was required to produce a certain grade of fluorescence or the appearance of the spectroscopic absorption bands, when it was added to a solution of zinc chlorid

Studenski added copper sulphate to the urine, precipitated with ammonium sulphate and dissolved the copper compound of urobilin in chloroform This color was compared with that produced by copper sulphate in a solution of urobilin of known strength

Ladage recognized the necessity of taking the urobilinogen into account and recommended that iodin should be added to convert it to urobilin The urobilin was then precipitated by saturation of the urine with ammonium sulphate, and the acid chloroform solution diluted until the spectroscopic absorption became invisible In Fr Muller and Huppert's method a mixture of barium chlorid and barium hydrate is added to the urine, and the precipitate is filtered off and washed with hot water The excess of barium in the filtrate is removed by sodium sulphate, and the filtrate after neutralization with sulphuric acid is saturated with ammonium sulphate The precipitated urobilin is filtered off, allowed to dry in the air and extracted three times after acidification with a warm mixture of alcohol and ether This solution is then evaporated to a convenient bulk and the amount of urobilin estimated spectrophotometrically

Charnas in 1909 gave us an alternative method to one based on the spectroscopic characteristics of urobilin, a gravimetric method in which after all urobilinogen in an ether extract of the urine has been converted into urobilin by exposure to sunlight, the urobilin is separated by water from the ether, and after filtration precipitated by ammonium sulphate, dried, dissolved in alcohol the alcohol evaporated *in vacuo* and the residue weighed

2 Spectroscopic Methods—Gerhardt (1889) and Beck (1895) made estimations by means of the spectrophotometer using Vierordt's tables of the grade of light extinction in different regions of the spectroscope in normal urine and solutions of urobilin

Saillet (1897) extracted the freshly passed urine with acetic ether In another specimen the urobilinogen was converted into urobilin by exposure to light and then extracted The two extracts are added together and diluted until the spectroscopic band disappears

Conner and Roper (1908) made approximate measurements of the amount of urobilin in urine by noting the number of dilutions required to obliterate the urobilin band in the urine They recommend the addition of a few drops of Lugol's solution to the urine in order to convert the urobilinogen into urobilin

Auche (1909) found that a solution of urobilin of 1 200,000 prepared by a method he describes is sufficient to make the five absorption bands of potassium permanganate all of equal intensity, although without it some are lighter than others He therefore superimposes the urine, which in some cases has to be extracted with a thymol-chloroform solution, over a solution of potassium permanganate, and dilutes the urine or its extract until all the bands are of equal intensity

Simpson (1910) acidifies the urine with sulphuric acid and exposes it to light for some time in order to convert urobilinogen to urobilin It is then diluted until the urobilin band disappears This method gave fairly accurate results when a dilution of fifteen or more volumes was necessary With smaller amounts the reading was apt to be obscured by other pigments

Hausmann (1913) adds copper sulphate to the urine and dilutes until the spectrum becomes invisible

Henocque, Hayem, Gautrelet, Deniqués, Hildebrandt, Riva and Zoja have used one modification or another of these spectroscopic methods

Charnas (1909) makes the urine alkaline, allows it to ferment at 37 C (98 6 F) for from twenty-four to forty-eight hours, acidifies with tartaric acid, and extracts with ether and petroleum benzin To a portion of the ether extract an ethereal solution of paradimethylamidobenzaldehyd is added and two to three drops of absolute alcohol saturated with hydrochloric acid gas After dilution if necessary with alcohol a spectrophotometric reading is made

3 Methods Depending on the Property of Fluorescence—Grimm (1893), Fischler (1906), Grigant and Monod (1909), and Descomps (1909) gage the amount of urobilin by the intensity of the fluorescence present in the zinc salt filtrate of the urine Various devices are adopted to aid the conversion of urobilinogen into urobilin

4 Colorimetric Methods—Bogomaloff (1892) used a colorimetric method depending on the depth of the red-violet color produced in a chloroform extract of urine containing urobilin by the addition of copper sulphate

Braunstein (1903) used a modification of this method

Flatow and Brunell (1913) estimate the amount of urobilinogen in fresh urine from the depth of the red color given on the addition of Ehrlich's reagent As a standard they use a phenolphthalein solution to which a few particles of metallic sodium have been added

Brugsch and Retzlaff (1912) modify Charnas' method by extracting the alkaline urine first with ligroin They make the final reading with Plesch's colorimeter, using a solution of Bordeaux red as the standard

The Stools

Quantitative estimations of urobilin in the stools have been carried out by comparatively few investigators Gerhardt at first used a somewhat complicated process of extraction and purification but later except in special cases simply extracted with acid alcohol and read spectrophotometrically Auche extracts with alcohol, removes other pigments by shaking with ligroin and thereafter makes the same spectroscopic determination as he employs in urine extracts

Simpson (1910) mixes the day's feces with water and adds enough dilute sulphuric acid to make the mixture distinctly acid For one or two days full exposure to light is allowed to convert the chromogen into urobilin Filtration is then carried out and the residue repeatedly extracted until the filtrate becomes colorless Direct examination of the filtrates is made and the amount determined from the degree of dilution required to obliterate the spectroscopic band

Brugsch and Retzlaff (1912) attempt to convert all the urobilin into urobilinogen by allowing the stools to stand for some time before extracting with ligroin to remove indol derivatives and then with acetic ether After the addition of paradimethylamidobenzaldehyd they make a colorimetric determination as in their method with urine

Serum

No quantitative work has been done with serum The methods employed for the detection of urobilin in serum were reviewed by Conner and Roper We shall refer therefore only to those which have appeared since their publication

Morel and Monod (1908) add alcohol to the serum and heat to boiling for half an hour The filtrate is concentrated and a drop of Obermeyer's reagent and an alcoholic solution of zinc acetate and acetic acid are added After twenty-four hours the filtrate is examined for fluorescence

Auche (1909) adds a solution of iodin in potassium iodid to the serum and afterward some zinc cyanate in ammonia and filters The filtrate is examined spectroscopically

Fromholdt and Nersessoff (1912) precipitate the oxalated plasma with alcohol, filter, concentrate, acidify, and shake with amyl alcohol, and then with alkaline water The water is then made acid and shaken with amyl alcohol and examined spectroscopically

III DISCUSSION OF METHODS

In reviewing the literature on these methods, it was evident that the one main difficulty encountered by all investigators was the problem of separating urobilin and urobilinogen from the other coloring matters present in the excretions without at the same time destroying more or less of it Many had frankly given up the attempt to attain any but the roughest purification, and had relied on the intensity of the physical properties of fluorescence or of light absorption In doing so they had, of course, confessed that their results were not quantitatively accurate, for variations in the impurities present would undoubtedly affect the basis on which their determinations rested Others have not remained satisfied with this, but have attempted to attain at any rate approximately pure solutions before estimation

The question as to which of these two methods produced the better result depended on whether more was gained in accuracy than was lost by destruction of urobilin in the various precipitations, separations and evaporations required

It appeared to us that there was one way in which the question could be answered When undiluted urine is examined spectroscopically, nothing is seen, as a rule, except a general absorption of light, which becomes deeper and deeper toward the blue end of the spectrum Even if urobilin is present, the absorption band cannot be clearly differentiated When the amount of urobilin, however, is much larger than the other light-absorbing substances present, one finds that on simple dilution with water the band between E and F becomes more and more distinct, so that it at last stands out sharply On still further dilution it begins to fade, and a more or less definite point is reached at which it becomes invisible when the full amount of light is allowed to pass through the spectroscope, but can still just be seen when the light is diminished In some urines one may have to carry the dilution to forty or more times the original volume of urine to reach this point At this dilution the disturbing effect of other substances is negligible Such urines afford an

excellent means of deciding as to whether any particular manifestation leads to a loss of urobilin or not, because the number of dilutions required to remove the urobilin band before and after the procedure can be very readily determined We were fortunate at this time in having under observation for several months a patient with bronzed diabetes in whose urine unusually large amounts of urobilinogen and urobilin were constantly present

Practical experience with this method very quickly showed us that any and every tried means which may be applied to separate urobilin from the urine was associated with more or less loss Extracts from precipitations with ammonium sulphate, separations by ether, chloroform or thymol-chloroform solutions, and processes of evaporation, with or without heat, all led to a greater or less diminution of the spectroscopic dilution value Such methods are applicable in the endeavor to obtain pure urobilin, but it seems to us that they can have no place in any quantitative estimation, for the amount destroyed is too variable and too incapable of control The one salient fact which has to be kept constantly in mind in choosing a method of estimation is that almost any manipulation is apt to be associated with more or less loss of urobilin It is better to recognize from the first that ordinary chemical methods are not applicable to a substance which is so labile, and that their employment gives only a fallacious appearance of accuracy Any gravimetric method for this reason seems to us to be entirely out of question Solutions of urobilin or urobilinogen may no doubt be obtained in a state of sufficient purity to admit of spectrophotometric determination, but with the means which have hitherto been employed for this purpose the error by loss so far outweighs any increase of accuracy in the final determination as to make results obtained by such methods of very doubtful value

Yet in the case of all urines in which the amount of urobilin was not exceptionally large and in dealing with stools, some preparation was necessary, for no direct reading could be made After trying a large number of procedures, the conclusion was reached that the most innocuous of all was to mix equal parts of urine and a saturated solution of zinc acetate in alcohol and to filter This is Schlesinger's qualitative test for urobilin A small amount of urobilin may be absorbed by the precipitate, and at first we filtered under pressure and washed the precipitate with alcohol, but latterly we have found that the error is so small compared with other unavoidable sources of error that in routine clinical examinations we have simply taken the first fraction of the filtrate

Another necessary procedure also soon became apparent It has of course, long been known that on adding acid to a urobilin solution the position of the absorption band is altered, but we had not gathered that the intensity was changed to the extent which we found in many urines

Sometimes the increase in the number of dilutions required to obliterate the band was ten times as great in filtrates which had been made strongly acid as in the fresh filtrate It was obvious that unless constancy could be attained in this point, no results of value could be obtained The intensity of the spectroscopic characteristics of urobilin are as dependent on reaction as is the property of fluorescence The difference is that to attain maximum fluorescence a nice balancing of reaction is required, whereas the strongest absorption band is obtained in a strongly acid solution and an excess of acid does no harm All that is required, therefore, is to be sure that enough acid is present For this reason alone the spectroscopic methods are to be preferred to those based on fluorescence in which large errors may arise from slight variations in reaction

Still another essential point in any method is that the urobilinogen as well as the urobilin shall be taken into account, and that if possible the quantitative relationship between the two bodies shall be established so that the result can be expressed as a single figure Urobilin is simply an oxidation product of urobilinogen, from the clinical or physiological points of view they have exactly the same significance Urobilinogen is potential urobilin In any specimen of urine or stool the relative amounts of the two are constantly changing, the amount of urobilin increasing and of urobilinogen decreasing the longer the exposure to light and air In some methods this difficulty is met by keeping the extract for twenty-four hours exposed to light, and it is assumed that all the urobilinogen will then have been converted into urobilin Others endeavor to hasten the process by the addition of oxidizing agents, and one method takes the opposite course, and by alkaline fermentation attempts to convert all the urobilin into urobilinogen

The only way by which urobilinogen can be determined, except gravimetrically, is by the spectroscope through the absorption bands of the pigment produced by its reaction with paradimethylamidobenzaldehyd We used a solution containing 20 gm in 300 c c of 50 per cent concentrated hydrochloric acid One c c was added to every 10 c c of the alcoholic zinc acetate filtrate This gave a sufficient amount of acid to produce the maximum intensity in the urobilin band and at the same time brought out the urobilin spectrum, so that the dilution values of both urobilin and urobilinogen could be read in the same filtrate The amount of urobilinogen could then be expressed as representing so many dilutions in the same way as with the urobilin

The first point investigated was the effect of light on urobilinogen It was found to vary greatly with the intensity of the light A few hours' exposure to direct sunlight suffices to cause the complete disappearance of urobilinogen With indirect light the process required a longer time, but after exposure to a diffuse light in a room with ground-glass win-

dows, there was found after twenty-four hours to be an almost complete disappearance of urobilinogen The figures in Table 1 show the effect of leaving the zinc acetate filtrates of urine in diffuse room-light for twenty-four hours

TABLE 1.—EFFECT OF LEAVING ZINC ACETATE FILTRATES OF URINE IN DIFFUSE ROOM-LIGHT FOR TWENTY-FOUR HOURS

Urobilinogen	
Filtrate Examined at Once	Examined After 24 Hours in Room-Light
32	1
37	0
45	2
25	0
21	1
12	0
7	0
16	0
34	0
40	1
18	1
25	0
19	0
40	0

The effect on urobilin of a similar exposure to light could not be determined in the same way, since all fresh urine and stools contain urobilinogen which passes over in part at least to urobilin It was invariably found, however, in these specimens in which by various means the urobilinogen had been removed, that light had a destructive effect on urobilin, as was shown by the diminution in the dilution value after exposure

Under the influence of light, as the urobilinogen diminishes the urobilin increases The question remained, however, as to how much loss of urobilinogen and urobilin occurred during this process The precise degree of loss could be found only if we knew the quantitative relationship between urobilinogen and urobilin and the relative light-absorbing powers of the urobilinogen and urobilin pigments These facts are not known, but an indication of the amount of loss of urobilin was gained by comparing the total dilution value obtained by adding together the urobilinogen and urobilin values in the fresh filtrates, in those which had been in darkness for twenty-four hours and in those which had been left in diffuse room-light for the same time

After twenty-four hours in darkness there had been a diminution of urobilinogen from 371 to 301 and an increase of urobilin from 6 to 134 The light-absorbing power of urobilin must then either be greater than that of the urobilinogen pigment, or one molecule of urobilinogen must break up into two or more molecules of urobilin In any case there is an increase of the total dilution values for urobilinogen and urobilin

together, associated with a diminution of urobilinogen and an increase in urobilin

TABLE 2.—QUANTITATIVE CHANGE IN UROBILINOGEN AND UROBILIN UNDER THE INFLUENCE OF LIGHT

Fresh Urine		After 24 Hours in Dark		After 24 Hours in Room-Light	
Urobilinogen	Urobilin	Urobilinogen	Urobilin	Urobilinogen	Urobilin
32	2	28	11	1	26
37	0	30	12	0	32
45	0	40	12	2	37
25	0	22	8	0	17
21	0	19	14	1	16
12	2	10	7	0	3
7	2	6	3	0	5
16	0	14	7	0	15
34	0	30	12	0	21
40	0	35	13	1	30
18	0	14	10	1	16
25	0	14	9	0	16
19	0	7	4	0	8
40	0	32	12	0	24
371	6*	301	134†	6	266‡

* Total "Urobilin" 371 + 6 = 377
† Total "Urobilin" 301 + 134 = 435
‡ Total "Urobilin" 6 + 266 = 272

In the filtrates exposed to light there is more than forty times as much urobilin as in the fresh filtrates and twice as much as in those kept in the dark, and yet in spite of that the total dilution value is much less, only 266 as compared with 377 and 435 If for a moment it is assumed that the diminution of urobilinogen and increase in urobilin in the filtrates kept in the dark represent the relationship between the two bodies, then 371 — 301 = 70 of urobilinogen should produce 134 — 6 = 128 of urobilin But the disappearance of 371 — 6 = 365 of urobilinogen in the filtrates exposed to light led to an increase of only 266 — 6 = 260 of urobilin instead of the 667 which should have resulted It is impossible to explain this decrease in total dilution value except as due to a destruction of part of the urobilinogen or urobilin or both Since the actual amount of urobilinogen or urobilin destroyed by exposure to light cannot be calculated, and varies so greatly with different degrees of intensity of light and with other unknown conditions, the conclusion was reached that it was not a means which could be used in any quantitative method to convert urobilinogen into urobilin, and that as far as possible exposure to light should be avoided in urobilin estimations

Attention was then turned to the effect of oxidizing agents which have been recommended especially by French investigators, but here

also the results were disappointing, for those reagents which were effective in oxidizing the urobilinogen were also very destructive to the urobilin

Charnas states that if urine is made alkaline and left for from twenty-four to forty-eight hours in the incubator, all the urobilin will be converted into urobilinogen, which can then be estimated by the spectrophotometer It is noteworthy that Charnas does not give any estimations indicating the decrease of urobilin and increase of urobilinogen during the process, nor does he bring forward any evidence to show whether or not any part of the urobilinogen or urobilin is destroyed by the fermentation which occurs It has long been known that in freshly passed urine urobilinogen is present alone or at any rate in far greater amount than urobilin, suggesting that as it is excreted through the kidneys it is all in the form of urobilinogen, and it has been the universal experience that the longer a urine is kept the less urobilinogen will be found in it, so that the statement that in alkaline urine left exposed to the air at a temperature of 37 C. the reverse process occurs, and that instead of an oxidation a reduction takes place, is not one which can at once be accepted We know that in the intestine there is a reduction of urobilin to urobilinogen, but there conditions are present much more favorable to reduction processes than those which are present in urine left exposed to air Outside the body the formation of urobilin constantly goes on, but to bring about the reverse process the strongest reducing agents we know have to be employed It seemed to us, however, that the question was one which could be decided by the simple spectroscopic method we have outlined

Readings of the relative amounts of urobilin and urobilinogen in thirty specimens of urine before and after alkaline fermentation at 37 C were made In most of the cases fermentation was allowed to continue for twenty-four hours The first twelve results are given in Table 3

TABLE 3—UROBILIN AND UROBILINOGEN BEFORE AND AFTER ALKALINE FERMENTATION AT 37 C

Before Fermentation		After Fermentation	
Urobilinogen	Urobilin	Urobilinogen	Urobilin
15	5	0	11
12	0	0	9
26	9	7	0
15	5	0	0
12	0	6	0
7	0	8	3
26	9	0	2
19	3	5	2
0	3	0	11
0	5	30	0
17	6	0	1
10	5	50	2

Of these thirty specimens there was a diminution instead of an increase of urobilinogen after fermentation in all except three In two there was a very considerable increase of urobilinogen, but both these specimens contained some bile, and as we found that urobilinogen was produced in decomposing bile we are inclined to ascribe these exceptions to this cause

It seemed that these results, so completely at variance with the statement of Charnas as to the effect of alkaline decomposition in urine, might be due to the urobilinogen having become absorbed in the heavy deposit of phosphates in the fermented urines The experiment was therefore repeated in ten urines, the readings before and after fermentation being made after the specimns which had been made faintly alkaline with ammonium carbonate had again been made acid with tartaric acid Before fermentation a total reading of urobilinogen 18 and urobilin 27 was made After twenty-four hours' fermentation, urobilinogen 13 and urobilin 16 was recorded, and after forty-eight hours, urobilinogen 12 and urobilin 4 We must conclude then that alkaline fermentation in the dark at a temperature of 37 C not only does not quantitatively convert urobilin into urobilinogen, but that it has a directly destructive action on both

Since there was no available way of converting all the urobilinogen into urobilin or vice versa, the only alternative was to make estimations of each separately But could such results be added together and expressed by a single figure? Our knowledge of the chemistry of these bodies is not yet sufficiently advanced to enable us to be sure that one or the other may not be a condensation of the other so that one molecule of urobilinogen, for instance, may break up into two or more molecules of urobilin No idea as to the relationship between them could be gained by noting the degree of increase in the dilution value of urobilin which followed a decrease in urobilinogen, for the factors concerned are too complex and variable to allow of any true idea being obtained in this way For while the urobilinogen is being converted into urobilin, both are undergoing other changes and are losing the spectroscopic characteristics on which our determinations rest In preparations of urobilin and urobilinogen made from bilirubin by Fromholdt's method we found such a lack of relation between the weight of bilirubin and the weight of urobilin produced, and such a variability in the relative amounts of urobilin and urobilinogen formed, that it did not seem likely that in this way either could we successfully determine whether or not urobilin and urobilinogen were equivalent in value In dealing with two such extremely unstable substances whose presence can be detected only by a single physical property and which are constantly changing into other bodies which we have no means of estimating, the problem of their

exact relationship is one whose solution will probably not be definitely established until we have more knowledge of the decomposition products of hemoglobin For urobilinogen and urobilin are only links in the chain of hemoglobin derivatives, they cannot be regarded as final end-products The problem which we were attempting to solve was not the exact quantitative determination of urobilin, however, but simply the question as to whether or not any information of clinical value could be gained from urobilin estimations, in spite of the errors necessarily involved in any of the present methods With this end in view the best that could be done was to add together the dilution values of urobilinogen and urobilin We do not know that they are equivalent, but, on the other hand, we know that they are not very different in value The proportion of the two substances varies so much in different specimens of urine, stool and bile that no comparison can easily be made if the dilution values for each are given separately

As a result of this preliminary review we determined that the best method would be one which avoided all but absolutely essential manipulation of the material, one which was based on the most characteristic of the properties of urobilin, the spectroscopic absorption bands, and one in which the attempt to convert all the urobilinogen into urobilin or vice versa was abandoned and the results of separate estimations of the two were simply added together Throughout this paper when the term "urobilin" is used apart from "urobilinogen," the sum of the dilution values of urobilin and urobilinogen is intended

The following are the details of the methods which we finally adopted

IV METHODS

Urine

The method of collection of the twenty-four-hour urine has a considerable effect on the total spectroscopic reading The vessel in which the urine is collected should be of dark brown glass and should be kept in darkness Thymol crystals should be added, for even in cases in which no obvious fermentation had occurred we sometimes found a diminution in the total amount if no preservative was present After measuring the amount of the twenty-four hour urine, 10 c c are mixed with 10 c c of a saturated alcoholic solution of zinc acetate, and after a few minutes filtered If a number of urines are being examined at the same time it is convenient to have test-tubes graduated to 10 and 20 c c Ten c c of the filtrate are taken and 1 c c of Ehrlich's solution[1] is added It was found that this amount produced a sufficient concentration of acid in the mixture to give the maximum intensity of the urobilin band and contained enough of the paradimethylamidobenzaldehyd for the reaction with urobilinogen The development of the urobilinogen band is not instantaneous We found that as a rule it had attained its full intensity in a quarter of an hour The action can be greatly accelerated by heating, but this is to be avoided It is better to wait for an hour before making the reading, and during this time the solution should be kept in

1 Paradimethylamidobenzaldehyd, 20 gm , concentrated hydrochloric acid, 150 c c , water, 150 c c

the dark After three or four hours there is a diminution of the urobilin and urobilinogen in filtrates from urines, so the estimation should be made not later than three hours after adding the Ehrlich solution We found that Citron's hand spectroscope[2] was the most convenient instrument to use The filtrate was washed into a graduate and diluted with tap water until first one and then the other bands of light absorption had disappeared when the full amount of light entered the spectroscope, but were still visible when the light was partly shut off This gives a fairly definite end point, and we did not find any great variation in the readings made by different persons It is important of course that the light shall be always of approximately equal intensity We made the readings in a dark room with a Tungsten electric bulb, holding the spectroscope close to the source of light In highly colored urines one may be in doubt as to whether or not a trace of urobilin is present, for there may be so much general absorption of light as to obscure the urobilin band in the undiluted filtrate There is no such difficulty with the urobilinogen band which lies between the red and yellow where there is no marked light absorption With urines containing bile, if the amount of urobilin is not very large, it is necessary to add some fullers' earth and to leave the mixture standing for some time before filtration If this is done the urobilin band can usually be read even in the undiluted filtrate The dilution required gives the value for 5 c c of urine If this figure is multiplied by the number of 5 c c quantities in the twenty-four hour urine, the number of dilutions which would have been necessary if all the urobilinogen and urobilin in the twenty-four-hour amount had been concentrated in a volume of 5 c c is obtained For instance, if in a twenty-four hour urine measuring 1,000 c c a reading of ten dilutions for urobilinogen and of twenty for urobilin were made, the total urobilin would be $30 \times 200 = 6{,}000$ We tried to determine the dilution value of definite weights of urobilin prepared from bilirubin, but different preparations varied so much in their dilution value that it was obvious that we were not dealing with pure urobilin and we abandoned any attempt to express our results in milligrams of urobilin

Stools

All the feces passed in the twenty four hours were collected in the same receiver, the stools being protected from light They were then washed into a large graduate and thoroughly ground up with water into a homogeneous paste, and water added to 0 5, 1, or if necessary 2 liters, depending on the quantity of stool After thorough mixing, 25 c c were taken and 75 c c of acid alcohol (95 per cent alcohol, 1,600 c c , concentrated hydrochloric acid, 25 c c , and water, 800 c c) were added The mixture was then put into a shaker for about half an hour A considerable number of extractives were tried for removing the urobilin from the stools, but none were found so efficient as alcohol with hydro chloric acid After thorough mixing in the shaker an equal quantity of a satur ated solution of zinc acetate in alcohol was added and the mixture was filtered After adding 2 c c of Ehrlich's reagent to 20 c c of the filtrate, the solution was put aside in a dark place until next day The addition of zinc acetate is not absolutely essential, but in some cases we observed an intensification of the uro bilin band following its use, and it is perhaps an advantage to make the urine and stool readings as far as possible under the same conditions The develop ment of the urobilinogen band was not always complete until six hours had elapsed, but thereafter there was no loss of urobilinogen or urobilin for a long time, although this was not the case with the zinc acetate filtrates before the addition of Ehrlich's solution The reading was made in the same way as with the urine and the total amount calculated for the volume of stool after grinding up with water, the dilution of the 25 c c by acid alcohol and zinc acetate being taken into account Instead of using tap water for dilution of the final extract, 60 per cent alcohol was required to avoid the development of a precipitate

2 This can be obtained from Paul Altmann, Luisenstrasse, Berlin, Germany

Bile

A measured amount of bile was mixed to a paste in a mortar with fullers' earth and left for some hours An equal amount of the alcoholic solution of zinc acetate was then added and the mixture filtered under pressure, and the remainder washed with measured amounts of zinc acetate alcohol In this way almost all of the bilirubin was absorbed, and though no doubt there was some loss of urobilinogen and urobilin, the comparatively small amounts found in the washings compared with the quantities present in the first filtrate seemed to indicate that the fullers' earth had very much less capacity for absorbing urobilin than bilirubin Small amounts of urobilin or urobilinogen could no doubt be overlooked by this method The Ehrlich solution was added in the same proportion as in the urine, but the dilutions had to be made with alcohol instead of water

Blood-Serum

We have tried various methods, but so far we have not been successful in demonstrating urobilin with certainty

V THE EXCRETION OF UROBILIN IN URINE

Urobilin is a normal constituent of the urine, but the amount is so small under ordinary conditions that it can be demonstrated only by extracting large quantities of urine

With the methods we have used, a positive result in twenty-four-hour specimens of urine indicates an abnormal increase over the amount usually present We have only very rarely been able to find a faint and doubtful degree of absorption in the region of the urobilinogen or urobilin bands in the urines of persons in apparent health When, therefore, urobilin is spoken of as being absent from urine, it is understood that an absence of an increased amount of urobilin is meant

Saillet found in two normal persons that the quantity of urobilin excreted during the eight hours of sleep was only about one-tenth of the total twenty-four-hour amount He gives two curves of the excretion during the daytime which show some relation to the body-temperature variations He suggests a connection with the processes of digestion and of general metabolism

In a case of bronzed diabetes with a large urinary excretion of urobilin, we had an opportunity to study the excretion from hour to hour The patient was on a von Noorden strict diet plus 50 gm of bread His habits were very regular He rose at 7 a m and went to sleep at about 9 p m Urine was passed every two hours during the daytime, and the amount of urobilin determined at once The amount passed during the six or eight hours of sleep was kept in the dark and the urobilin determined at the earliest opportunity In such freshly passed specimens, practically all the urobilin is in the form of urobilinogen, and the probable error arising from the assumption that the urobilinogen and urobilin readings represent equivalent values is avoided For over a month, the two-hourly excretion was determined every day, with the result that there

was invariably found to be a marked diminution in the amount excreted at night A graphic representation of the excretion during three consecutive days is given in Chart 1 The chart is typical of the results throughout the whole period It shows the decrease of urobilin in the night urine and the great variation in the quantity from day to day and from hour to hour The only constant feature is the fall during the night Otherwise there is complete irregularity and the hour of maximum elimination occurred at any time during the day

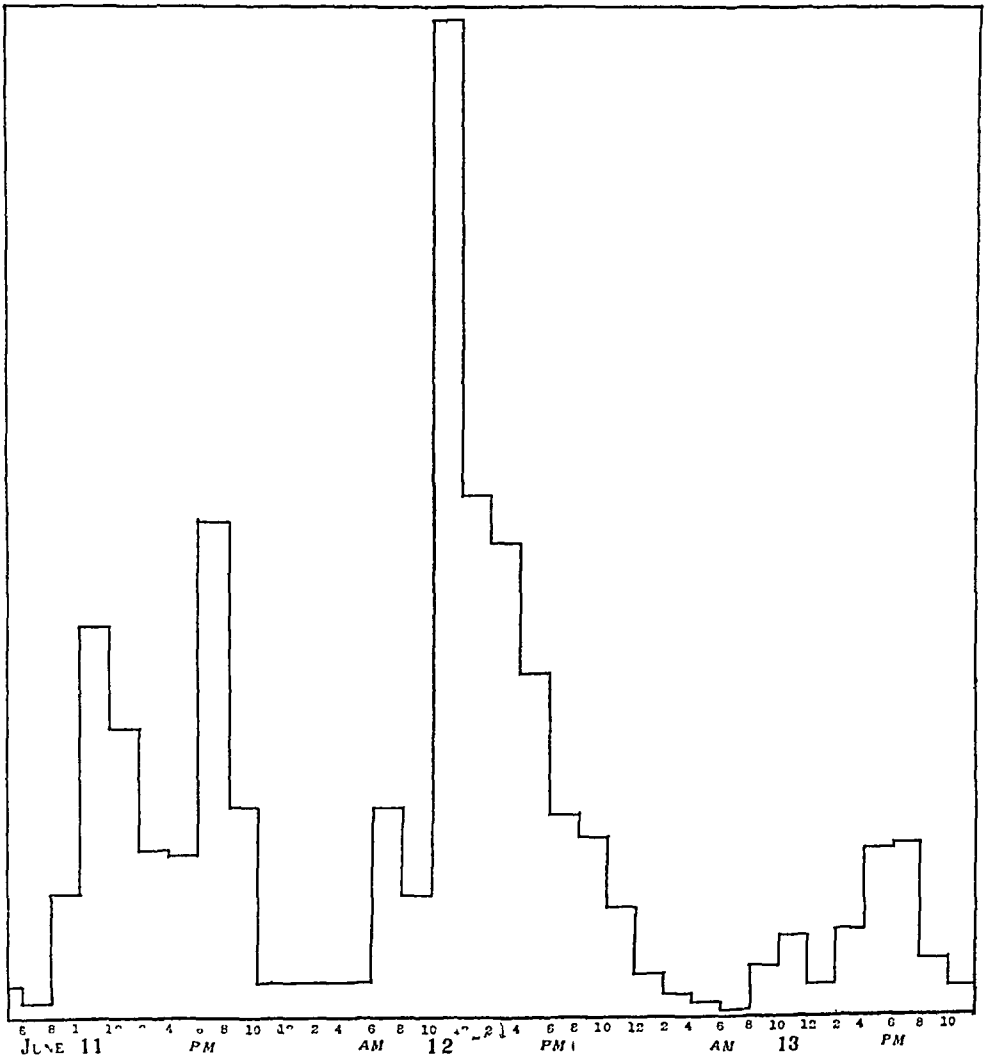


Chart 1 —Excretion of urobilin, June 11, 12 and 13

When the patient remained up and took his meals during the night and slept in the daytime, the amount excreted in the night was much larger than in the day

Charts 3, 4, 5, 6 and 7 show the relation between the excretion of water, total solids, sugar, chlorids and urea and urobilin The relation was only a very general one, for the urobilin excretion shows irregular and wide fluctuations

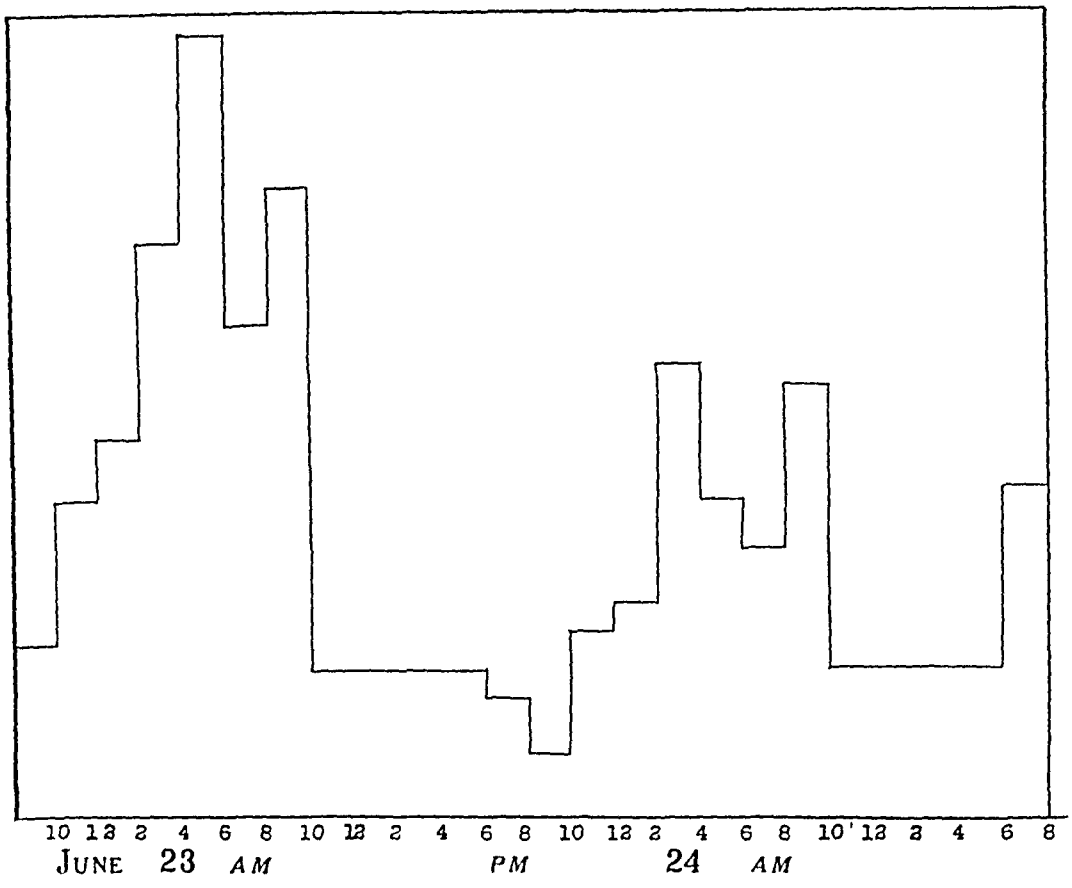


Chart 2.—Excretion of urobilin, June 23 and 24

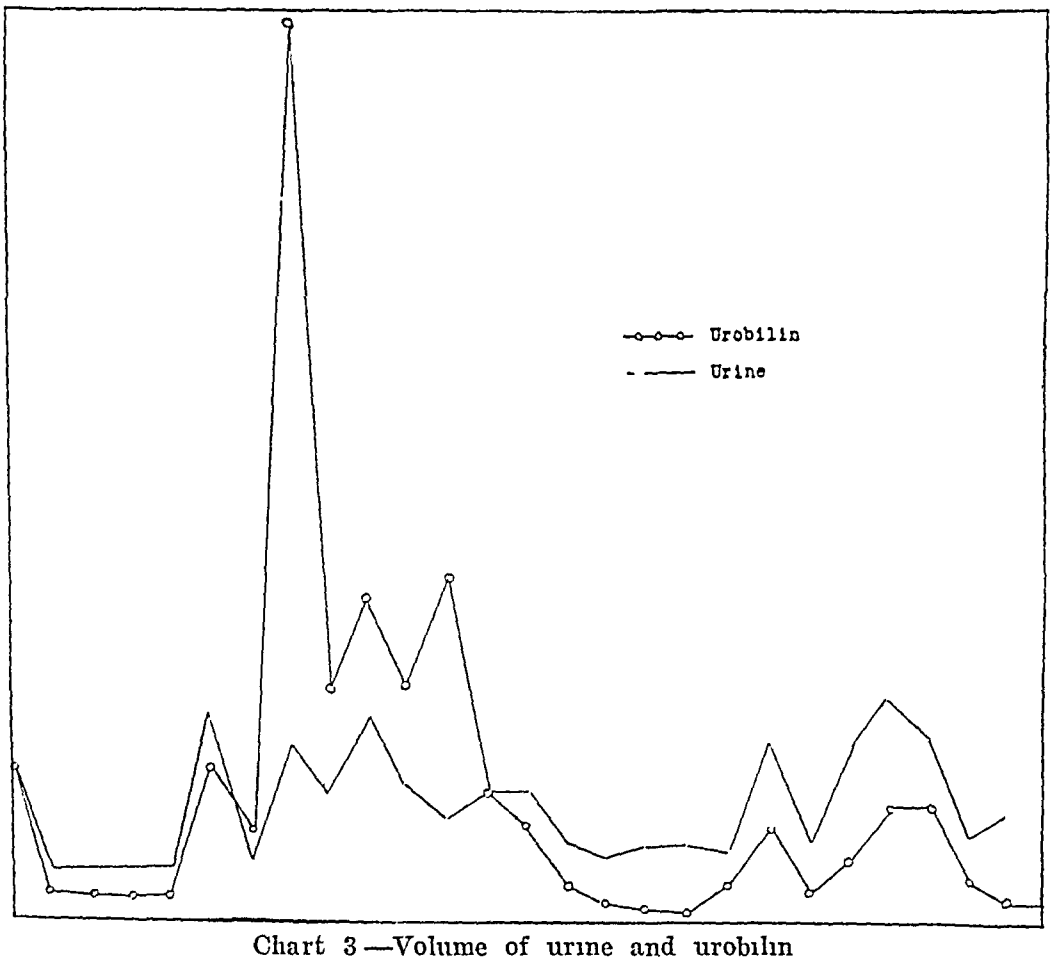


Chart 3.—Volume of urine and urobilin

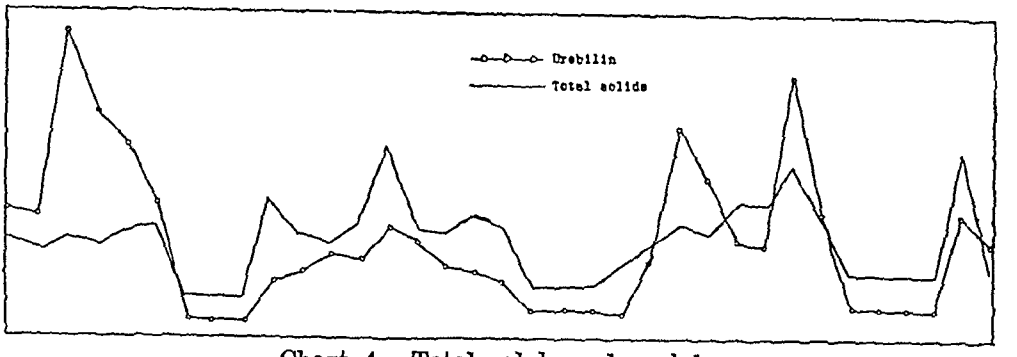


Chart 4—Total solids and urobilin

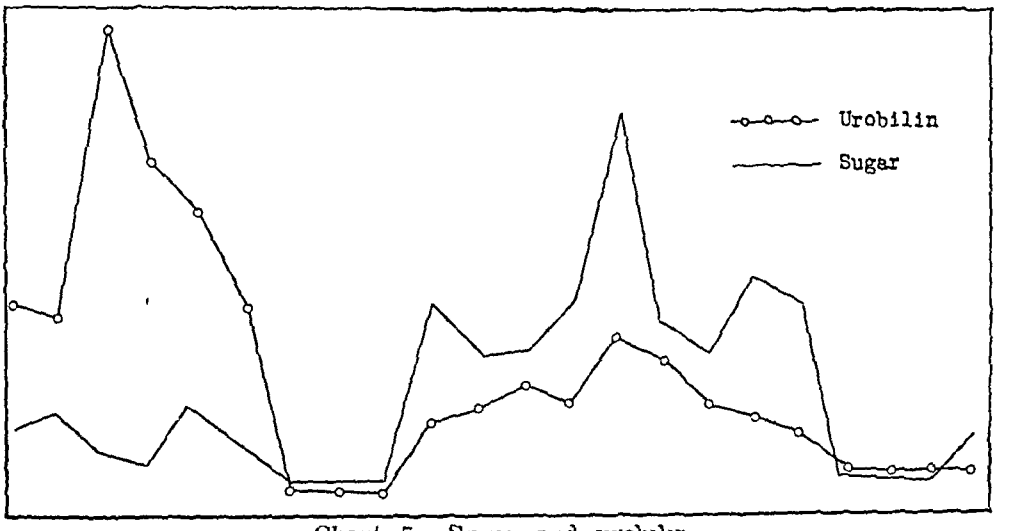


Chart 5—Sugar and urobilin

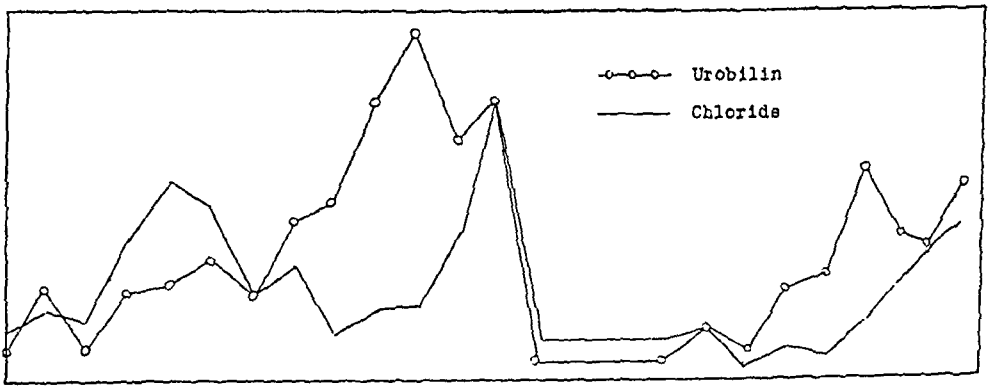


Chart 6—Chlorids and urobilin

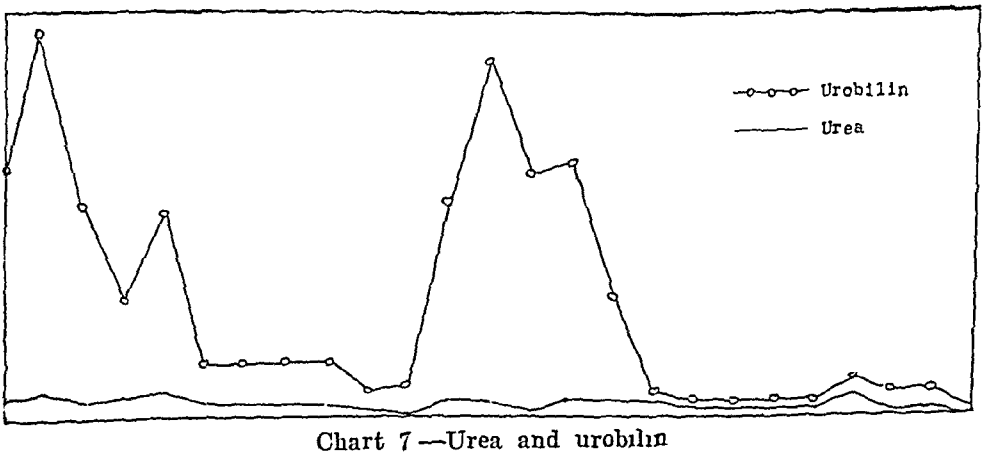


Chart 7—Urea and urobilin

These results appear to us to be of interest in connection with the question of urobilin excretion by the kidneys It is generally agreed that most of the urobilin is formed in the intestine and is carried by the portal vein to the liver There it is supposed to be either broken down further or resynthesized to bilirubin or hemoglobin (Brugsch) except for a very small part which is carried into the general circulation and is excreted through the kidneys When the liver is diseased, the destruction or rebuilding of urobilin fails more or less, so that a greater quantity of the urobilin absorbed from the intestine passes by the liver to the kidneys

The wide fluctuations in the quantities of urobilin in this case can scarcely be due to variations in the amount of absorption from the intestine It is true that bile enters the duodenum only at intervals, but they are regular intervals when the periods of digestion are regular, and on these charts no signs of any periodicity during the daytime can be traced In any case, the bile passes through the small intestine in about four hours, and as our findings as to the amount of urobilin in various parts of the intestinal tract show, the greatest quantity of urobilin is in the large intestine, not in the small Urobilin is always present in the large intestine, and from this situation one could not expect any considerable changes from hour to hour in the amount absorbed Urobilin is a readily diffusible substance, and since water is removed from the large intestine, it seems probable that urobilin will also be taken up by the blood

Nor does it seem that changes in the excretory functions of the kidneys can account for more than a small part of the urobilin variations The amounts of sugar and chlorids fall during the night mainly because their absorption from the alimentary tract almost ceases and not because there is any marked decrease in the eliminating functions of the kidneys But urobilin is present in the large intestine in as large amount during the night as during the day, and if absorption is uniform one would expect uniform excretion, or at least no greater variation than might be ascribed to alterations in the circulatory and nervous mechanism of the kidneys We have no evidence of any specific diminution in excretory power of the kidneys for any substance during the night

If the absorption and excretion of urobilin appear to be too continuous and uniform to explain these charts, can we regard the variations shown in them as indicative of fluctuations in the power of the liver to keep the urobilin reaching it from the intestine from entering the general circulation?

The patient had an extremely large liver which post mortem was found to be cirrhotic and to contain a large amount of the pigment found in cases of hemochromatosis During the day, when the work of the liver was increased because of the absorption of food products, it might

be imagined that the liver would be less able to cope with the urobilin brought to it, and the great increase during the daytime might be thus explained But the wide and irregular fluctuations from day to day and from hour to hour, in spite of the fact that the meals were taken at regular intervals, remain to be accounted for Possibly some other factor such as a formation of urobilin in the diseased liver may have played a part In Saillet's two curves of hourly urobilin excretion in people in health, no such wide variations are to be seen

Other possible factors which may play a part in alterations of the urinary excretion of urobilin are the changes which urobilin undergoes in the blood and tissues The form in which urobilin circulates in the blood-stream is still unknown, and the question as to whether it may not there in part be broken up or synthesized to some other substance, or may not be stored in some form or other in the tissues, is still unsolved, and it is possible that in them an explanation may be found for these anomalies of urobilin excretion At present, they can only be recorded

VI THE EXCRETION OF UROBILIN IN THE STOOLS

In ten adults in whom there was no reason to suspect any disturbance of hemoglobin metabolism, the average daily excretion in the stools was worked out These cases, as shown in Table 4, showed variations between 3,307 and 8,737, and a general average of 6,475

TABLE 4.—AVERAGE DAILY EXCRETION OF UROBILIN IN STOOLS OF TEN ADULTS WITH NORMAL HEMOGLOBIN METABOLISM

Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin in Stools
8	Inguinal hernia	8,685
15	Fractured femur	3,855
3	Chronic appendicitis	6,080
3	Endometritis	3,307
6	Neurasthenia	4,887
10	Neuritis	8,702
15	Chronic arthritis	6,604
11	Neurasthenia	8,737
6	Fractured femur	5,840
6	Fractured tibia	8,053

The impossibility of gaining any idea as to the amount of urobilin excreted in the stools by an examination of a single twenty-four-hour collection is apparent when the figures for the daily excretion of any of the cases we have worked with are examined (Table 5) The variations, it is seen, are sometimes very large indeed

To a certain extent, of course, this is accounted for by differences in the quantity of feces passed from day to day, but there are certainly other factors involved We were particularly struck with the fact that on the days following a period of twenty-four hours during which no stool was passed, there was not as a rule any increase in the urobilin If

the urobilin in the stools retained in the large intestine were absorbed by the blood-stream, one would have expected an increase in the urobilin in the urine on these days But on reviewing the figures, no constant increase in the urobilin in the urine could be established We were then inclined to attribute this apparent loss of urobilin to destruction within the intestine, but when the effect of keeping stools in an incubator at 37 C was tried, we found to our surprise that there was no less marked loss of urobilin

TABLE 5—UROBILIN EXCRETED IN STOOL

Day	Urobilin in Stool			
1	2,408	4,800	16,640	5,120
2	10,880	3,360	5,280	13,440
3	*	3,840	7,680	2,560
4	7,360	960	1,440	*
5	4,480	*	4,800	3,840
6	1,280	6,080	15,360	14,400
7	7,680	10,240	25,920	*
8	4,000	8,000	8,320	35,200
9	*	*	3,840	1,536
10	720	3,200	5,760	3,200
11	7,040	11,200	6,720	7,040

* No stool

The stools were thoroughly ground up in water and kept in the incubator Every day, 25 c c were removed and extracted with acid alcohol With the exception of the last stool, the figures show only such differences as lie within the limits of error of the method (Table 6) There was only a slight diminution of urobilinogen and increase of urobilin[3]

TABLE 6—EFFECT ON UROBILIN OF KEEPING STOOLS IN INCUBATOR

No	Fresh Stool	After 24 Hours	After 48 Hours	After 72 Hours	After 96 Hours
1	15	16	16		
2	3	3	2	3	3
3	12	19	14	18	11
4	57	62	65	66	65
5	20	21	20	18	24
6	60	55	48	53	60
7	3	3	3	4	4
8	65	60	55	51	48

So long then as stools are kept from exposure to light there does not seem to be a very rapid loss of urobilin Nor, on the other hand, is there any increase such as might have occurred if the formation of urobilin in the freshly passed stool had been incomplete

The absence of great destruction of urobilin in stools kept under these conditions outside the body does not, of course, preclude the possibility

3 Later experiments showed that there was a gradual diminution of both urobilin and urobilinogen, so that after a week about a quarter of the original dilution value had been lost The greater constancy in the experiment given here was due to concentration by evaporation

of such an action taking place within the bowel, where absorption and excretion are proceeding and where there is no exposure to air It was thought that an indication of such a loss of urobilin either by absorption or destruction was obtained when the relative amounts of urobilin present in the contents at different levels of the intestinal tract were compared

The feces obtained at post-mortem in five cases were ground up in so much acid alcohol as gave, so far as could be seen, an approximately equal consistency The relative amounts of urobilin were as follows duodenum, 1, jejunum, 1 2, ileum, 4 8, cecum, 12 3, descending colon, 5 5

The relative decrease in the descending colon as compared with the cecum certainly suggests a loss of urobilin, but the number of cases examined was too small to permit of any definite conclusion In all probability, both absorption and destruction are at work The absence of increased urobilin in the urine on the days of constipation is not by any means conclusive evidence against the occurrence of absorption of urobilin from retained stools, for the absolute want of any relationship between the amount of urobilin in the stools and in the urine is a fact which must impress any one who makes such concomitant observations No doubt the quantity in the intestine is a factor in determining the amount excreted by the urine, but except under conditions such as an unusually large hemoglobin destruction, when there may be a very great increase of urobilin in the stools, its effect is altogether overshadowed by other influences at work on the urobilin in its passage through the blood-stream, liver, bile, tissues and kidneys

The interpretation of the results of urobilin estimations in the stools is a comparatively simple matter when compared with the complexities and obscurities surrounding the question of the elimination of urobilin in the urine, and we believe that from a clinical point of view, work on the stools will give results of much greater value and of much less doubtful significance than has been the case up to the present, when the attention of clinicians has been directed almost exclusively to the urine

Our own experience in cases of pernicious anemia, and the reports of Simpson in cases of malaria have convinced us that, if constipation or diarrhea are avoided, the determination of the average stool urobilin over a period of several days is the best guide we have to the quantity of hemoglobin which is being broken down in the body Its importance in anemias and in all cases in which excessive blood destruction is suspected does not need to be elaborated

VII THE EXCRETION OF UROBILIN IN BILE

Urobilin has been found in human bile post mortem by Jaffé, Muller, Tissier, Gerhardt, Beck and Braunstein After the introduction of Ehr-

lich's reagent, urobilinogen was found in forty-three of forty-six cases by Kimura Neither is constantly present, for in cases in which severe diarrhea had occurred, or in which there had been obstruction to the flow of bile into the intestine, they were not found Austin and Ordway in a very careful study found urobilin present in 94 per cent of fifty cases with biliary fistulas in which urobilin was present in the stools and urine In seven cases in which no urobilin could be demonstrated in either the stool or urine, urobilin was present in the bile in two Fischler found urobilin in the bile of normal dogs After establishing a biliary fistula and obstructing the common bile-duct, urobilin disappeared from the bile, but it was still found, though in diminished amount, in the stools When the liver was damaged by amyl alcohol, urobilin appeared in considerable amounts in the bile He believes that the only way in which this can be accounted for is by assuming that the urobilin was formed in the liver itself

Our experience with bile removed post mortem goes to show that the amount of urobilin is extremely variable In Table 7 the value of the urobilin in 10 c c of bile is given in sixteen cases

TABLE 7.—UROBILIN IN BILE REMOVED POST MORTEM

		Dilution Value in 10 c c Bile
1	Pernicious anemia	3,940
2	Pernicious anemia	80
3	Pneumococcal meningitis	2 100
4	Pyonephrosis	4,500
5	Bronchopneumonia	650
6	Streptodiplococcal septicemia	0
7	Cerebral embolism	620
8	Aneurysm	300
9	Aortic incompetence	45
10	Diabetes	200
11	Bronzed diabetes	1,800
12	Carcinoma of liver (without duct obstruction)	45
13	Tetanus	0
14	Carcinoma of larynx	0
15	Hypophysis tumor	0
16	Adherent pericarditis and valvular disease	0

Of these sixteen cases there were five in which no urobilin was found by the method we used But if larger amounts of bile had been used for extraction we might have found traces, and we therefore cannot say more than that the quantity was at least very small No relation was found between the length of time between death and examination of the bile and the amount of urobilin found In one case urobilin to a dilution value of 1,800 for every 10 c c of bile was present, although it was removed from the body within three-quarters of an hour after death It does not seem that post-mortem decomposition of bilirubin can possibly explain the variations in quantity which were found

On the other hand, there is no obvious connection between the nature of the disease and the amounts obtained We are inclined to believe that the degree of absorption of urobilin from the intestine just before death is an important factor In the two patients with pernicious anemia, estimations of the amount of urobilin present in the intestinal contents were made, and an enormous quantity was found in the case which contained so much urobilin in the bile, and comparatively little in the one which showed a low dilution value In another case in which severe diarrhea had completely emptied the intestine, no urobilin could be detected in the bile In a dog which died four days after the common bile-duct had been severed and in whose intestines no urobilin was found there was also no urobilin in the bile, although it was found in the bile of normal dogs These observations bear out the well-recognized importance of the intestinal source of biliary urobilin, and yet exceptions are sufficiently striking to make one hesitate to accept the exclusively enterogenous origin of urobilin

In a few cases with biliary fistulas after operations for gall-stones the urobilin in the bile was found to increase as the bile began again to enter the intestine, but there was no definite qualitative relationship between the amount in the bile and in the stools An exception was found in one patient whose case is discussed later, in whom urobilin was present in large amount in the bile while urobilin was practically absent from the stools This was a case of gall-stones in which for a period of eight days after a gall-bladder fistula had been established, the total urobilin in the stools amounted to only 720 dilutions, while in the bile an amount equivalent to 35, 144 dilutions was found The stools contained no more urobilin than we found in a case in which there was undoubtedly a complete occlusion of the common duct Probably no bilirubin at all was entering the intestine Later on when the bile again passed through the common duct, the urobilin increased in amount in the stools to 33,216 dilutions in seven days, but the percentage of urobilin in the bile, which still came from the fistula for the first four days of this period, was only 230 dilutions for each hundred c c of bile, in contrast with 1,360 when there was practically no urobilin in the stools In cases of this sort it is difficult to avoid the conclusion that the urobilin has been formed in the liver itself

VIII UROBILIN IN SERUM

Urobilin is present in the intestine and is excreted in part by the kidneys It presumably reaches the kidneys by means of the blood One would expect to be able to detect it in the blood of those not infrequent patients in whom even more urobilin is found in the urine than in the stools Yet there is no point in the whole subject on which there is more diversity of results and of opinions The constant absence of uro-

bilin from the serum in cases of urobilinuria was a main support of the theory advanced by Gilbert and Herscher that urobilin was formed from bilirubin during excretion through the kidneys Even long after the inadequacy of this hypothesis had been exposed by the work of Muller and the intestinal origin of the urobilin of the urine had been generally accepted, no one was able to demonstrate the presence of urobilin in the blood or serum Muller and Gerhardt believed that it was not present in appreciable amount in the blood, and Schlesinger reported fifteen cases of very marked urobilinuria in which the serums gave a negative reaction for urobilin

It was not until 1906 that Huber published a report in which he stated that urobilin was always found in serum when urobilin was present in the urine He ascribed the negative results of Schlesinger to the fact that he had not added Lugol's solution to the zinc acetate filtrate in order that urobilinogen might be oxidized to urobilin But considerable doubt was cast on Huber's results when it was pointed out that the green fluorescence characteristic of urobilin might be closely imitated by biliverdin, and that especially after the addition of Lugol's solution, the bilirubin normally present in serum might be oxidized to biliverdin Biffi (1907) extracted oxalated blood with chloroform, examined the filtrate spectroscopically and found urobilin present in small quantities in a number of cases and stated that in lobar pneumonia it was almost constantly found Moller could not confirm Biffi's findings as to the relative frequency of a positive reaction for urobilin in serum, but with zinc acetate and Lugol's solution found it in only two cases, both lobar pneumonias, and later with Biffi's method in two other cases, pneumonia and one of pernicious anemia

Further evidence of the occurrence of urobilin in serum was given by von Jaksch, who stated that in fatal cases of pneumonia the serum separated from blood removed by venesection showed a green fluorescence which disappeared on exposure to light Conner and Roper (1909), in a study of the relation between the bilirubin in serum and urobilin in the urine, repeatedly and carefully examined the serums of over sixty patients with urobilinuria, with uniformly negative results, until the serum of a patient dying of lobar pneumonia was encountered This serum gave a markedly positive reaction for urobilin with all the methods In nine other cases of pneumonia shortly before death the serum contained large amounts of urobilin In three severe cases of pneumonia in which the patients recovered, urobilin was detected in small amount in the serum, but in fifteen other cases in which the patients lived, no urobilin was found There was no parallelism between the amount of urobilin in the serum and in the urine

Lehndorf (1913) could find urobilin in only two of the serums which he investigated One was from a case of decompensated mitral stenosis and the other from a case of myocardial degeneration with pleurisy shortly before death Fromholdt and Nersessoff (1912) by a somewhat complicated method and by extracting relatively large volumes of oxalated plasma, were able to demonstrate urobilin in the serum of seven out of eleven cases with urobilin in the urine They record a doubtfully positive reaction in a case of occlusion of the common duct with no urobilin in either the stools or the urine The spectroscopic band of urobilin became unmistakable after the addition of iodin, but they are inclined to think that this may possibly have been due to a conversion of bilirubin into urobilin

Hildebrant (1909), who, after many negative results, found the spectrum of urobilin in the serum in a case of pneumonia shortly before death, believes that urobilin normally circulates in the blood as urobilinogen, because in this case the urobilin band appeared only after the serum had been exposed for some time to light and air He obtained a red color on mixing Ehrlich's reagent with the serum before the urobilin had appeared Moller, though he is inclined to agree with Hildebrandt, points out the technical difficulties of urobilinogen recognition in serum, since the hydrochloric acid precipitates the proteins He obtained an apparently positive reaction in only two of his cases In three cases Conner and Roper showed the characteristic red color and confirmed the presence of urobilinogen by finding the absorption band of the benzaldehyd compound But in the other cases in which urobilin was present they did not find any urobilinogen

Conner and Roper's spectroscopic recognition of urobilinogen is the only observation which can be taken as approaching to a proof of the presence of this body in serum, and in view of the substances of the indol group which may give a spectrum very similar to that of the urobilinogen compound, their statement cannot, in the absence of any data as to the position of the absorption band, be taken as decisive

In fact, the evidence so far points in just the opposite direction and seems to indicate that urobilin, instead of circulating in the reduction form of urobilinogen, is present as an oxidation product which does not possess the spectroscopic characteristics of urobilin and so escapes detection This evidence is all the stronger because it has been collected independently by three observers since 1903, none of whom apparently knew about the work of the others Finding no urobilin in the blood of cases in which he expected it, Schlesinger tried the effect of adding urobilin to serum and to blood He discovered that whereas it was readily demonstrated in the serum, it disappeared in the whole blood or in serum to which red blood-cells had been added Clarens made the same

observation and in 1911 Roth and Herzfeld confirmed Schlesinger's experiment and suggested that the disappearance of the urobilin might be due to oxidation

They found that oxygenated serum destroyed the urobilin and that in whole blood its disappearance might be delayed by carbon dioxid In a few cases, though not in all, they were able to demonstrate the presence of urobilin in serum after it had stood for from twenty-four to forty-eight hours mixed with finely powdered zinc Since urobilinogen is found in the urine of patients in whom no urobilin can be found in the serum, it seems that the kidneys must possess the faculty of reducing the oxidized urobilin They were able to carry out only two experiments with kidney extracts, one of which gave a definite production of urobilin in a serum in which it had previously been found absent It is noteworthy that urobilin was formed with an alcoholic kidney extract, so that the process cannot depend on any vital activity in the cells

The conception of the oxidation of urobilin in the blood-stream is strengthened when it is remembered that the only cases in which urobilin has been with certainty found in the serum are those in which there was a great reduction in the available oxygen of the blood They have been almost without exception cases of lobar pneumonia shortly before death

The effect on urobilin of the blood and tissues opens up a new field for investigation and it is not improbable that along these lines facts may be discovered which will throw light on the present obscurity

IX ANIMAL EXPERIMENTS

The Excretion of Urobilin in Animals

No satisfactory estimations could be made on the stools of rabbits or goats because the amount of urobilin was too small

The same difficulty, though to a lesser extent, was encountered in dealing with the excreta of cats and dogs Even when the extract was made as concentrated as possible it seldom required more than a few dilutions to obliterate the spectra of urobilinogen and urobilin We have not been able to find any systematic investigation of the pigments of the stools of dogs or cats, but it is known that cholecyanin,[5] a derivative of bilirubin which is not regularly present in human stools, is constantly found Possibly the greater part of the bilirubin in the intestine of these animals may be excreted in other forms than urobilin Certainly the acid alcohol extracts of the stools contained a large amount of light-absorbing material, so much so as to render the urobilin band somewhat obscure at the low dilution at which it had to be read And not infrequently other absorption bands were seen The method did not then give such definite and clear results as were obtained with human stools

5 Fischler Inaug Diss, Heidelberg, 1906, p 84

It was soon found that such small amounts of urobilin as were detected varied considerably from day to day No satisfactory explanation for this variation was obvious, but one possible factor appeared to be the length of time during which the feces remained in the intestine Hard constipated stools as a rule contained very little urobilin The following experiment shows how markedly the quantity of urobilin increases during regular action of the bowels as compared with a period of constipation

Dogs B, C and D were normal Dog A had had a biliary fistula and a closure of the common bile-duct At the time of this experiment the fistula was closed and the common duct was again patent Urobilin was present in the urine and was added to the amount in the stools The figures (Table 8) represent the total dilution value of the urobilin excreted during ten days In the first period the dogs were constipated, in the second sufficient cascara was given to obtain a stool every day without giving rise to marked diarrhea

TABLE 8.—UROBILIN IN CONSTIPATION AND IN REGULAR BOWEL ACTION

	First Period of Ten Days		Second Period of Ten Days	
	Urobilin	Number of Stools	Urobilin	Number of Stools
Dog A	5,640	4	13,120	10
Dog B	14,400	8	19,200	10
Dog C	9,200	5	16,420	10
Dog D	12,860	7	37,200	9

In these four dogs the total urobilin during the period of constipation was 42,100 and in the period during which cascara was given, 85,940, more than twice as much During another ten-day period castor oil was given, and the total amount passed was 51,250, but regular daily movements were not obtained Constipation, therefore, in dogs diminishes the amount of urobilin in the stools We are satisfied that this is true for man also though we cannot bring forward any systematic evidence The diminution may be due either to increased absorption of urobilin from the intestine or to destruction of the urobilin If increased absorption were the cause, the excretion of urobilin in the urine should be increased In normal dogs or cats urobilin was never found in the urine in sufficient amount to estimate But in Dog A, in which the common duct had been tied and a biliary fistula made, it was found six months later that communication between the bile-ducts and the intestine had become reestablished and that the fistula had closed In this animal considerable amounts of urobilin were constantly present in the urine The cause of this was found at death to be an extremely marked cirrhosis of the liver, which had developed probably as a result of infection through the fistula combined with the initial bile stasis

TABLE 9.—UROBILIN IN URINE AND IN STOOL OF DOG A

Date	Urobilin in Urine	Urobilin in Stool
Dec 2	160	No stool
Dec 3	248	800
Dec 4	192	800
Dec 5	224	No stool
Dec 6	21	400
Dec 7	100	No stool
Dec 8	160	No stool
Dec 9	120	No stool
Dec 10	240	No stool
Dec 11	400	1,200
Dec 12	520	No stool
Dec 13	240	No stool
Dec 14	280	800
Dec 15	80	No stool
Dec 16	200	800
Dec 17	600	No stool
Dec 18	160	No stool
Dec 19	680	400
Dec 20	480	No stool
Dec 21	500	No stool

Increased absorption during constipation might be expected to show itself in a rise of urobilin in the urine in this case Yet we were not able to establish any such increase in urinary excretion The average amount of urobilin in the urine on the seven days in which urobilin was lost from the body in the stools was 273, and 272 on the thirteen days during which urobilin was retained in the intestine This evidence would have been more convincing if we had succeeded in contrasting a period of regular evacuation with a period of constipation, but on giving cascara it was found to be impossible to be sure of excluding contamination of the urine with the stools Still if the figures for the four days from December 3 to December 6, inclusive, during which three stools were passed, are contrasted with those from the 7th to the 10th when there were none, it is seen that a total of 685 dilutions of urobilin were present in the first period, and only 620 when the bowels did not act at all

It seems probable, therefore, that destruction of urobilin plays a much larger part than increased absorption in the diminution of urobilin which occurs during constipation This is only what would be expected of a substance which outside the body is so readily altered

Diarrhea, unless it is so pronounced as to lead to the excretion of unaltered bile, does not seem to have nearly so great an influence in diminishing the quantity of urobilin

The total excretion of urobilin per kilogram of body-weight in dogs which had been kept under exactly the same conditions and on the same diet for a long time was determined over a period of forty-one days (Table 10)

TABLE 10.—EXCRETION OF UROBILIN PER KILOGRAM OF BODY WEIGHT

	Weight, Kg	Dilution Value Per Kg
Dog C	7 82	6,100
Dog B	9 95	5,220
Dog D	17 80	5,450

Dog A, with cirrhosis of the liver, which was also kept under the same conditions for the same time, and which during that period exhibited no obvious signs of ill health, weighed 4 02 kg and excreted urobilin with a dilution value of 8,806 per kilogram body-weight This is an increase of 58 per cent over the average excretion of urobilin in the three normal dogs It does not seem probable that an increased hemoglobin metabolism could account for this It appears much more likely that there was either a diminished destruction of urobilin or an increased formation of urobilin from bilirubin Constipation was present to a more marked degree in this dog than in the others, so that the increase of urobilin was probably even more marked than the figures suggest A diminished destruction, if such occurred, must have operated in the body itself If one of the functions of the liver is the synthesis of urobilin absorbed from the intestine into bilirubin, an explanation of the increase in urobilin excretion is afforded by the supposition that the diseased liver in this case allowed much of the absorbed urobilin to pass through it into the general circulation to be excreted in the urine But the possibility of the increase in urobilin being due to a formation of urobilin from bilirubin in the diseased liver cannot be excluded Outside the body we found that urobilinogen and urobilin were produced in considerable amounts in bile left at a temperature of 37 C And it seems possible that such a breaking down of bilirubin may occur in stagnating bile especially if an infection of the bile passages exists

In attempting to follow the effect of closure of the common bile-duct in dogs on the urobilin excretion, a difficulty was encountered in connection with the methods we used In the alcoholic stool extracts a pink color was produced which apparently showed the spectroscopic absorption of urobilinogen That in reality it was some other substance was shown by the difference in the color reaction with Ehrlich's solution and also by the fact that on exposure to light of the zinc acetate filtrate the absorption band still persisted instead of disappearing In all probability this substance was indol or some derivation of indol Von Moracewski found that in alcoholic stools there is an increase in indol which has an absorption band very similar to that of urobilinogen

To avoid confusion with this substance we have taken no account of the urobilinogen either before or after the closure of the common bile-duct This makes the total amounts given inaccurate, but our object

was to see whether or not the absence of bile from the intestine would completely stop all excretion of urobilin As is seen from the two experiments reported below, urobilin disappears for a time, but it afterward returns in diminished though relatively not inconsiderable amount in the stools In both these cases also urobilin after an interval was found in the urine The impossibility of obtaining urobilin-free stools in dogs even though all bile is prevented from entering the intestine has been noted by Fischler Brugsch and Yoshimoto found that urobilin reappeared after an interval in the stools of a dog with biliary fistula

OBSTRUCTION OF COMMON DUCT

Dog E—Section of Common Duct Removed

Icterus, acholic stools and bile in the urine appeared At the post mortem a distended gall-bladder and ducts were found No communication with the intestines A few patches of organized lymph were found on the liver and adhesions round the resected duct but no general peritonitis Urobilin given as total amounts in periods of six days (Table 11)

TABLE 11—Total Urobilin, Dog E, in Periods of Six Days

Period	Stools	Urine
BEFORE COMMON DUCT CLOSURE		
First	3,200	0
Second	2,320	0
AFTER COMMON DUCT CLOSURE		
Third	0	0
Fourth	512	185
Fifth	32	56
Sixth	320	0
Seventh	160	76

Dog F—Section of Common Bile Duct Removed

Uninterrupted recovery with development of jaundice At the post-mortem the gall-bladder and ducts were found to be greatly distended There was no communication between them and the intestine The urobilin is given as a total of estimated dilution value for the stools and urine passed in periods of six days (Table 12)

TABLE 12—Total Estimated Dilution Value of Urobilin, Dog F, in Periods of Six Days

Period	Stools	Urine
BEFORE COMMON DUCT CLOSURE		
First	9,312	0
Second	5,120	0
AFTER COMMON DUCT CLOSURE		
Third	0	0
Fourth	0	0
Fifth	576	396
Sixth	2,368	1,084

BILIARY FISTULA WITH OBSTRUCTION OF COMMON DUCT

In three other dogs the common duct was obstructed and a biliary fistula established No jaundice developed

Here also the amount of urobilin in the stools decreased, but did not disappear Urobilin appeared in the urine of Dog A, which was afterward found to have

developed a cirrhosis of the liver In the other two it was absent The urobilin is given as the total dilution values of six-day periods (Table 13)

After death no communication was found between the bile passages and the intestine in Dogs G and H

TABLE 13.—TOTAL DILUTION VALUES OF UROBILIN, DOGS G, H AND A, IN PERIODS OF SIX DAYS

	Period	Stools	Urine*
		BEFORE OPERATION	
Dog G	First	1,920	0
	Second	1,590	0
	Third	3,524	0

* Urobilin was occasionally found in the urine, but this was due to contamination with bile It was at first difficult to prevent the urine wetting the dressings and taking up some of the bile which was rich in urobilin

		AFTER OPERATION	
Fourth		240	0
Fifth		1,845	0
Sixth		560	0
Seventh		384	0
		BEFORE OPERATION	
Dog H	First	1,744	0
	Second	930	0
	Third	632	0
		AFTER OPERATION	
Fourth		64	0
Fifth		176	0
Sixth		688	0
		BEFORE OPERATION	
Dog A	First	3,480	0
	Second	3,256	0
		AFTER OPERATION	
Third		728	0
Fourth		520	22
Fifth		280	68
Sixth		1,260	26
Seventh		632	239
Eighth		720	

The presence of urobilin in the stools after closure of the common bile-duct cannot be explained as due to the excretion of bilirubin into the intestine from the blood, for it was also found in the stools of the dogs in which the bile was allowed to drain out of the body and in which there was no increase of bilirubin in the blood The only possible explanation seems to be that urobilin itself was excreted from the blood into the intestine Where was this urobilin produced? Urobilin is known to have formed in old hematomas in various parts of the body, so that the formation of urobilin from bilirubin in the tissues of the icteric dogs is a possibility But the fact that it also was found in the excreta of dogs which were not jaundiced points to the liver as the place

of formation, for in the dogs with biliary fistula this was the only organ in which bile was present Urobilin was found in the bile escaping from the fistulas

We must conclude then that in dogs the intestine is not the only site of origin of urobilin, but that under certain conditions urobilin is formed in the liver Whether or not this is a normal function of the liver is another question which will be discussed later

These experiments, though few in number and inconclusive in their results, are yet sufficient to show the complexity of the subject and the number of factors which may play a part in excretion of urobilin Variations in the amount of formation of urobilin in the intestine or in destruction after it has been formed, the degree of absorption from the intestine, the fate of the absorbed urobilin in the liver, whether it is rebuilt to bilirubin or is allowed to pass into the general circulation, the question as to a change of bilirubin into urobilin in the liver under certain conditions, and the possibility of the excretion of urobilin from the blood into the intestine are some of the points which have to be considered in interpreting the results

In any case we must conclude that in dogs at least the theory of the exclusively enterogenous origin of urobilin does not suffice to meet the facts

X CLINICAL REPORTS

To enumerate all of the conditions in which urobilin has been found in excess clinically does not seem to us desirable as we wish to point out the significance of its occurrence in the few in which its clinical study has been found to be of most value The following clinical reports are selected from a series of observations made by us during the last two years

Diseases of the Liver without Much, if Any, Interference with the Entrance of Bile into the Intestine

TABLE 14.—SUMMARY OF CASES 1-6

No	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin Urine	Stools
1	I N	53	Portal cirrhosis	572	3,647
2	A F	10	Portal cirrhosis	241	3,088
3	C D	14	Cirrhosis (syphilitic)	456	3,451
4	C B	30	Bronzed diabetes	5,800	7 400
5	F M	4	Hanot's cirrhosis	2,034	3,360
6	F O'B	15	Abscess of the liver	1,713	4,047

CASE 1.—I N, enlarged liver with ascites Red blood-cells about 3,000,000 and hemoglobin 55 per cent Remained during the time of observation in about the same condition Fluid was removed from the abdomen on several occasions Urobilin was never found in it There was a slight grade of jaundice, the serum on one occasion showing six times the normal amount of bilirubin One month after the estimations were discontinued the patient died and the liver showed a well-marked portal cirrhosis Chart 8 shows the urinary and stool excretion

of urobilin The stools were given as averages for periods of three days to diminish variations due only to changes in the motor activity of the colon and rectum The considerable variations in the quantities in the stools and the fact that urobilin was not constantly found in the urine are the noteworthy points in this case

CASE 2.—A. F. A typical case of portal cirrhosis

CASE 3.—C. D. Small liver, large spleen, hematemesis melena Glycosuria with polyphagia, polydipsia and emaciation Positive Wassermann

CASE 4.—C. B. Very much enlarged liver and spleen Ascites Pigmented skin Glycosuria At post-mortem the diagnosis of bronzed diabetes was confirmed by the finding of a deeply pigmented cirrhotic liver and pancreas Chart 9 shows the great variations in the amounts of urobilin in the urine

CASE 5.—F. M. A man aged 48 complaining of persistent slight jaundice of two years' duration No loss of weight. No pain General health good Liver greatly enlarged, extends 10 cm. below costal margin Spleen much enlarged, projects 12 cm. below costal margin No ascites Urine contains bile Red blood cells 4,400,000 Hemoglobin 86 per cent Negative Wassermann

TABLE 15.—DAILY UROBILIN IN CASE 5

Date	Urine	Stools
November 23	1,300	4,800
November 24	2,950	5,120
November 25	1,104	960
November 26	2,784	2,560

CASE 6.—F. O'B. Admitted with a temperature and polymorphonuclear leukocytosis, complaining of pain and tenderness in the right hypochondriac region On the eighth day after admission the abdomen was opened and a large abscess of the liver drained

In this group of cases the urobilinuria is evidently not due to an increased formation of urobilin in the intestine, for the quantities found in the stools are not above the normal limits There is no evidence of any overflooding of the liver by urobilin derived from an increased destruction of hemoglobin That the abnormal quantity of urobilin in the urine is to be ascribed to the pathological changes in the liver which were present to a marked degree in all these cases is suggested not only by the constancy of the association of urobilinuria with liver disease which has been noted by all observers, but also by the fact that in particular cases it is possible to observe the diminution and final disappearance of urobilin from the urine in those cases in which an abnormal condition in the liver is removed The case of F. O'B. with a large liver abscess was a good example of this During the week preceding the opening of the abscess the average daily excretion was 2,187 For a few days after the operation the quantity in the urine remained high and then dropped so that the daily average for the seven days after operation was only 244

It will be noted that it is not possible to exclude pronounced disease of the liver by a failure to find urobilin in the urine on one examination As may be seen from the chart of the case of I. N. (Chart 8, Case 1), who had a very marked cirrhosis of the liver, there was a period of five days during which no urobilin could be detected And even in the

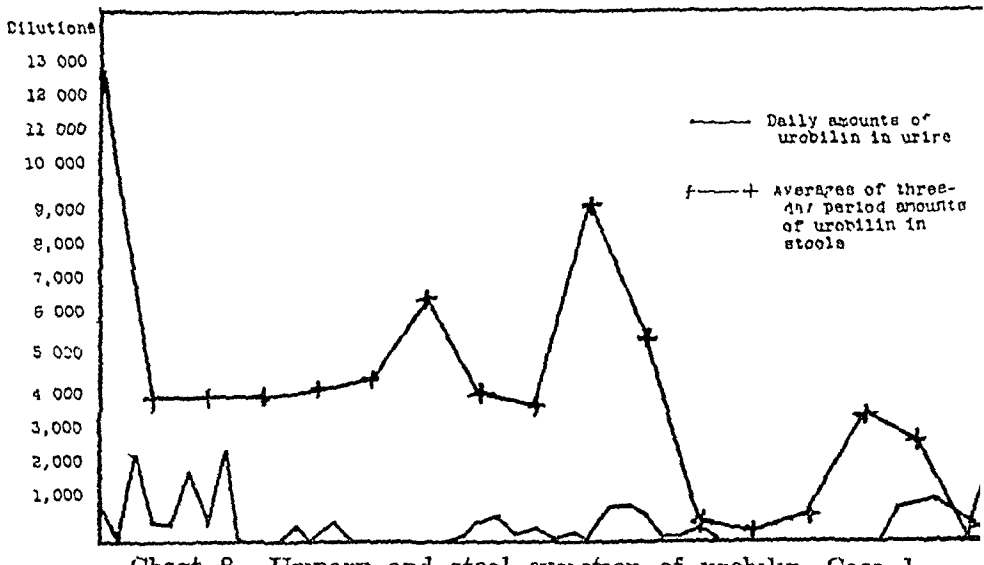


Chart 8.—Urinary and stool excretion of urobilin, Case 1

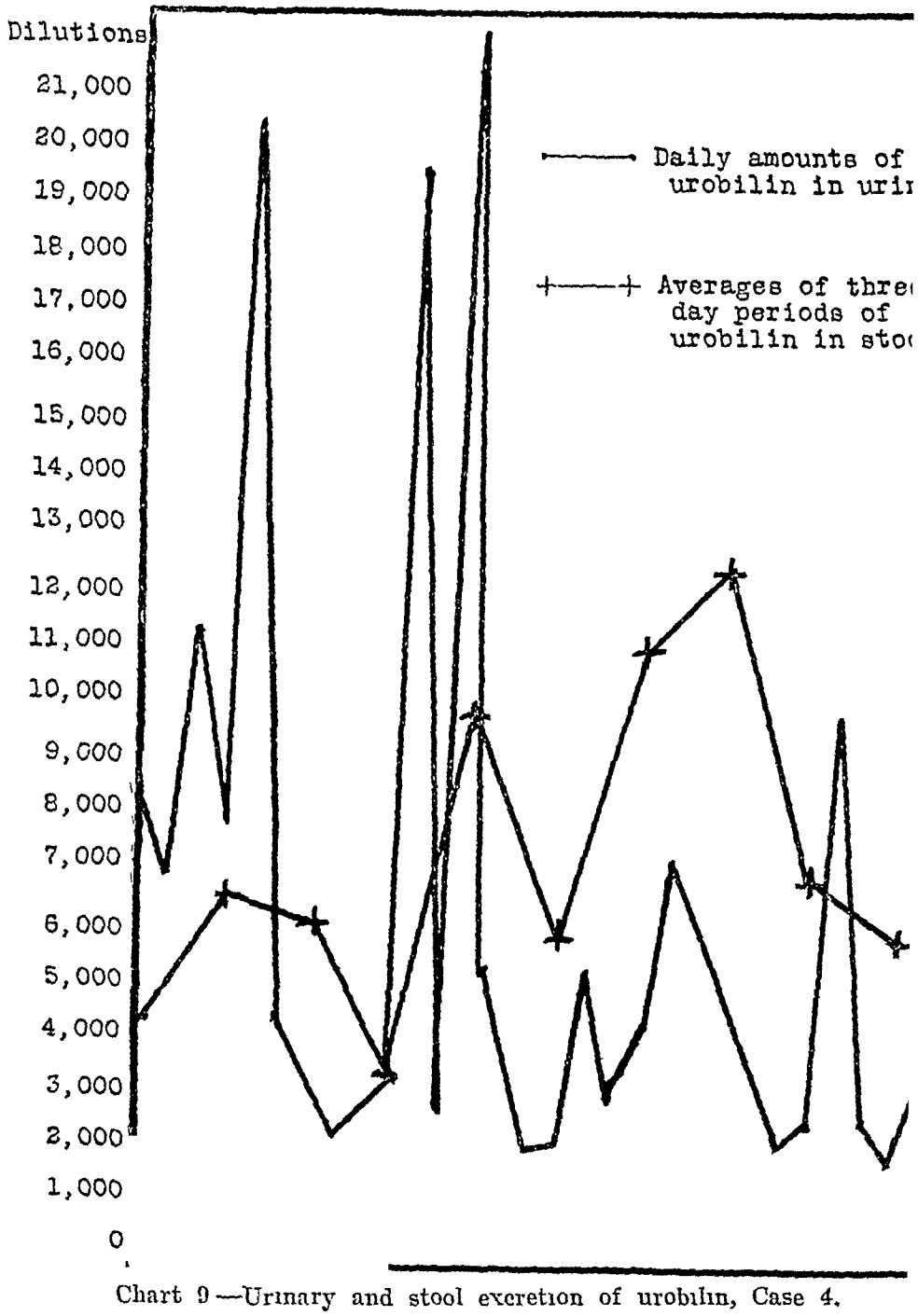


Chart 9.—Urinary and stool excretion of urobilin, Case 4.

patient C B, in whose urine there was as a rule such a large amount of urobilin, none was found in one twenty-four-hour specimen The fluctuation in the quantity of urobilin from hour to hour and from day to day makes it imperative to take the average of a series of twenty-four-hour specimens before an idea can be obtained as to the grade of urobilinuria in any particular case

The study of a large series of cases of liver disease along these lines, with concomitant estimations of the urobilin in the stools and in association with other tests of hepatic efficiency, will be necessary before it is possible to say whether or not the degree of urobilinuria may be taken as an approximate guide to the amount of impairment of liver function

Cases in Which Part of the Bile Entered the Intestine and Part Left the Body through a Gall-Bladder Fistula

TABLE 16.—SUMMARY OF CASES 7 AND 8

No	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin		
				Bile	Urine	Stools
7	A R	18	Cholelithiasis	788	0	4,240
8	B H	13	Chronic cholecystitis	1,505	0	2,669

CASE 7.—A R This patient was operated on for relief of dyspeptic symptoms No icterus A thickened gall-bladder containing a large stone was found The gall-bladder was drained There was no jaundice following the operation

CASE 8.—B H Operated on for symptoms of dyspepsia No jaundice before or after operation A moderately thickened injected gall bladder with adhesions was found and drained

In these cases there was no obstruction to the flow of bile through the common duct, and only a part of the bile escaped from the fistula

The urobilin found in this fraction of the bile is of interest in connection with the question as to how much of the urobilin is simply excreted again into the intestine after absorption by the portal blood-vessels To judge from these two cases, it would appear that a very large part of the absorbed urobilin is excreted into the intestine unchanged But it must be remembered that neither of these was a normal case, and that the possibility of the formation of urobilin in the liver itself existed

Complete Obstruction to the Entrance of Bile into the Intestine

TABLE 17.—SUMMARY OF CASES 9 12

No	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin	
				Urine	Stools
9	F W*	34	Carcinoma of hepatic ducts	0	178
10	G W	5	Carcinoma of stomach	0	0
11	A M	6	Carcinoma of pancreas	0	0
12	B M	3	Carcinoma of duodenum	0	0

* Thirty-five gm of dried bile were given to this patient by mouth in an attempt to confirm Muller's experiment in which he succeeded in producing urobilinuria in such cases No urobilin appeared in the urine, but this may have been due to the form in which the bile was given Fromholdt also obtained negative results with various preparations of bile and found that it was necessary to give fresh bile as Muller himself did Some of the urobilin found may have been derived from bile given by mouth, but it was also found before it was taken and also more than two weeks afterward

CASE 9.—F W This was a case which we have already reported in which there was a large liver, deep jaundice and clay-colored stools At the post-mortem a large carcinomatous mass in the liver was found to have completely obstructed the hepatic duct

CASE 10.—G W Jaundice had been present for two weeks before the estimations were made Afterward, at operation a pyloric carcinoma obstructing the common duct was found

CASE 11.—A M Deep jaundice of several months' duration Post mortem a scirrhous carcinoma of the pancreas was found

CASE 12.—B M At operation, a carcinoma of the duodenum was found which completely obstructed the ampulla of Vater

In these four cases, no bile was entering the intestine except such traces as might be excreted from the blood Bile was present in all the tissues of the body, and practically the only mode of excretion was through the urine

The law of the enterogenous theory of the origin of urobilin, that without the entrance of bile into the intestine, no urobilin is formed, is borne out by the last three cases but not by the first

In itself, we should not have been inclined to attribute much importance to this exception, if it had not been that Fischler and we ourselves had found urobilin in the stools of dogs with closed common ducts, and that several observers (Gerhardt, Hoppe-Seyler, Tsuchuya and Fischer and Meyer-Betz) have even found urobilin in the urine when no bile was entering the intestine

Our method gives positive results in the urine only when the amount is distinctly larger than normal, so that urobilin may very well have been present And it is noteworthy that we found urobilin in the fluid removed post mortem from the broken-down cancer tissue in the liver

In the other three patients in whom no urobilin could be detected in the stools, the liver itself was not directly involved It seems, then, that this case gives some evidence in support of the hypothesis that the diseased liver may itself form urobilin

Cases in Which there was Obstruction to the Entrance of Bile into the Intestine and in Which Part of the Bile Left the Body through a Gall-Bladder Fistula

TABLE 18.—SUMMARY OF CASES 13 AND 14

No	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Bile	Urine	Urobilin Stools
13	A R	5	Cholelithiasis	0	0	0
14	E W	8	Cholelithiasis	5,856	640	160

CASE 13.—A R Admitted with typical signs of acute cholecystitis Slight icterus but no bile in the urine The gall-bladder was drained For the first few days the jaundice deepened and bile appeared in the urine Thereafter, it diminished and on the fifth day after operation the urine was free from bile On the seventh day no icterus could be detected

CASE 14.—E. W. The patient was operated on for symptoms of gall stones. There was no jaundice. A stone was removed from the common duct and the gall bladder drained. After the operation there was deep jaundice, acholic stools and bile in the urine.

In both of these patients the manipulations during the operation caused a closure of the common duct, and the drainage through the fistula was not sufficiently free to prevent the occurrence of jaundice. There was, therefore, a complete or almost complete obstruction to the passage of bile into the duodenum combined with the escape of a part of the bile through the gall-bladder fistula.

Though clinically so similar, there is nevertheless, a remarkable contrast in the urobilin figures. The patient A. R. might be cited as an excellent example in support of the purely enterogenous origin of urobilin, while the patient E. W. appears to offer very strong evidence in support of the possibility of the formation of urobilin in the liver under certain conditions.

The subsequent course of urobilin excretion in these two cases appears to be worthy of mention, and for this purpose it is most conveniently divided into periods corresponding with changes in the biliary excretion.

In Case 13, during the days immediately following the operation, when bile was still present in the intestinal tract, considerable amounts of urobilin were found in the urine and some in the bile (Table 19, first period). Thereafter, during the time in which there was jaundice, acholic stools and bile in the urine, urine, no urobilin was found either in the bile or urine (Table 19, second period).

In the third period, the jaundice diminished, the stools became colored, and with this urobilin reappeared in the bile and urine.

The fourth period seemed to illustrate the effect of recovery of the liver function, for as the patient's condition improved, the urobilin was found to diminish and at last disappear from the urine in spite of the fact that more bile was entering the intestine because of the removal of the gall bladder drain.

TABLE 19.—UROBILIN IN CASE 13.

Period and Date	Bile	Urine	Stools
First	432	1,270	*
Second	0	0	0
Third	492	1,240	5,896
Fourth —			
April 1	144	704	0
April 2	18	864	1,600
April 3	†	656	7,680
April 4		448	8,960
April 5		0	7,680
April 6		0	6,400
Fifth —			
May 1	0	0	160
May 2	0	1,224	240
May 3	0	3,900	64
May 4	84	4,480	‡
May 5	36	7,800	19,540
May 6	120	5,040	6,784
May 7	224	1,700	9,230

* No stools.
† Drain removed.
‡ Stool lost.

Three weeks later the patient returned with recurrence of the cholecystitis and at least a partial blocking of the common duct On May 1 the fistula was reopened and much bile, mucus and pus escaped Urobilin, present at first in very small amounts in the stools, increased in the next few days and urobilin reappeared in the urine and bile (Table 19, fifth period) During the whole of the next month during which the patient was under observation, urobilin was present in considerable amount in the urine, as if during this second attack, the liver had suffered some more lasting damage Subsequently, though sometimes found in small amounts, it was often absent

This case, therefore, supports what may be called the orthodox theory of the necessity of the presence of bile in the intestine for the production of urobilin, and also may be taken as illustrating the effect of damage to the liver on the urinary excretion of urobilin

On the other hand, Case 14 cannot be reconciled with the exclusively enterogenous origin of urobilin and is the clearest instance we have encountered of the necessity of admitting that, at any rate in some cases, urobilin may be produced elsewhere than in the intestine

During a time when the stools were colorless, the skin jaundiced, and the urine laden with bile, that is to say when everything pointed to the practical absence of bile from the intestine, there was a very large excretion of urobilin in the bile and a not inconsiderable amount in the urine

TABLE 20.—UROBILIN IN CASE 14

Period	Bile	Urine	Stools
First	5,856	640	160
Second, (average daily excretion)	1,176	592	3,625

During the next eight days, urobilin reappeared in the stools and the jaundice diminished, but the urobilin in the bile and urine, instead of increasing as one might have expected, decreased in amount

It seems most probable that in this case the urobilin in the urine during the first period was absorbed by the blood-stream from the urobilin in the bile and excreted through the kidneys The diminution during the second period, in spite of the reentrance of bile into the intestine, may perhaps be ascribed to a decrease in the formation of urobilin by the liver, as it began to recover its normal functions In any case, the large amount of urobilin in the bile cannot have been absorbed from the intestines

If, in such cases as these, a marked urobilinuria may be present in spite of the total or almost total absence of urobilin from the intestine, it would seem to be likely that in all cases of urobilinuria in liver disease, some part at least of the urobilin has an extra-intestinal origin

CASE 15.—A H Pernicious anemia During the period in which estimations were carried out, the red blood-cells varied from 1,300,000 to 1,800,000 and the hemoglobin from 31 to 38 per cent

CASE 16.—A T Pernicious anemia The red blood-cells numbered 1,000,000 and the hemoglobin was 28 per cent

Diseases Associated with Changes in Hemoglobin Metabolism

TABLE 21.—Summary of Cases 15-19

No	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin Urine	Stools
15	A H	25	Pernicious anemia	470	24 977
16	A. T	12	Pernicious anemia	1 058	22 014
17	E P	4	Secondary anemia from intestinal hemorrhage	0	2,400
18	L S	4	Primary polycythemia	1,849	3 240
19	H L	7	Secondary polycythemia from asthma and emphysema	806	10,011

Case 17.—E P For eleven years the patient had had bleeding from the bowel The stools were sometimes tarry, sometimes contained bright red blood No cause was found even after the abdomen was opened At the time of observation the red blood cells varied from 2,100,000 to 2 550 000 and the hemoglobin from 27 to 29 per cent Hemorrhage ceased after a Whitehead operation and the patient entirely recovered

TABLE 22.—Urobilin in Case 17

Date	Urine	Stools
November 23	0	5 120
November 24	0	*
November 25	0	2 560
November 26	0	1 920

* No stool

Case 18.—L S Noticed a bluish tinge in her face and hands three years ago During the time of observation the red cells numbered from six and one-half to seven and one half millions and the hemoglobin was over 110 per cent No normoblasts were found There was no splenic enlargement

TABLE 23.—Urobilin in Case 18

Date	Urine	Stools
September 5	4 455	800
September 6	1 560	8 960
September 7	1 080	3 200
September 8	360	*

* No stool

Case 19.—H L Asthma and emphysema since childhood Marked and constant cyanosis At the time of observation there were 7 350 000 red blood cells, and 120 per cent of hemoglobin There was no fever and the general health of the patient was as good as it ever had been

TABLE 24.—Urobilin in Case 19

Date	Urine	Stools
September 2	980	8 000
September 3	2 016	14 080
September 4	0	11 520
September 5	864	8 000
September 6	0	9 600
September 7	900	4 480
September 8	880	14 400

Pernicious Anemia.—The amounts of urobilin in the stools of these two patients on whom a careful study was made are much higher than we have found in any other condition, although in neither of them was there any acute blood crisis during the time of observation The

quantity in the urine was not very great, but in two other patients from whom the stools could not be obtained for examination, very large amounts were found, corresponding to 4,895 and 16,800 dilutions

That the increase of urobilin in the urine is in the main due to an overburdening of the liver with an excessive amount of urobilin absorbed from the intestinal tract, and not to a primary functional deficiency of the liver, is most probable

The marked increase of urobilin in the stools is of special interest as evidence of a distinction between those forms of anemia associated with a great increase in hemoglobin metabolism, such as the cases of pernicious anemia we have cited and others, such as the secondary anemias following hemorrhage and carcinoma, in which the low urobilin content of the stools indicates a diminished destruction of hemoglobin

Secondary Anemia.—It is especially in cases of secondary anemia that combined urobilin estimations in the urine and stools ought to afford interesting and valuable results

The case of secondary anemia from hemorrhage which is given above affords a striking contrast when compared with the cases of pernicious anemia Other cases in which secondary anemia was a complication of some other condition such as carcinoma, showed a diminution rather than an increase in total urobilin excretion

On the other hand, the case of H D, who had a diplostreptococcal endocarditis, may be cited as an example of an anemia due to increased blood destruction During the first twenty-six days the average excretion in the stools was 12,841, twice as great as the normal average, and there was a dilution value of 781 in the urine During the next seventeen days there was a fall in the stool excretion to 7,801 and in the urine to 659, although there was no increase in the percentage of hemoglobin or red blood-cells The estimation of red blood-cells and hemoglobin can give no adequate guide as to the total turn-over of hemoglobin in the body, but an approximation to this may be obtained by urobilin determinations in the urine and stools In cases of anemia of unknown origin, the finding of an increased urobilin excretion might be of diagnostic importance in directing attention to the search for some active blood-destroying agent as the cause

Polycythemia.—The case of primary polycythemia gave unexpected results The urobilin in the stools was low instead of being increased, and at the same time there was a very considerable amount of urobilin in the urine, although there was no evidence of any liver disturbance The finding of urobilin in the urine of the patient with secondary polycythemia was also unexpected

Both these patients were markedly and constantly cyanosed, and when the connection between cyanosis and the presence of urobilin in

the serum is recalled, the question rises as to whether, under normal conditions, a part of the urobilin absorbed from the intestine may not escape the liver and be changed in the blood, except under certain conditions, such as a diminution in the percentage of oxygen, when it might be excreted in the urine

Unfortunately, we have not had an opportunity of following up this point in other cases with cyanosis

Miscellaneous Cases

In a large number of patients with various disorders, no pathological increase in the urobilin in the urine was found

In some, however, who showed no evidence of gross anatomical disease of the liver or of great blood destruction, considerable amounts of urobilin were present in the urine One of these, a case of alcoholism, is of interest

The patient was 59 years old and had suffered from attacks of acute gout for forty years There were large urate deposits round the joints At the time of observation, he was recovering from a prolonged spell of drinking, and was in a condition of great prostration with complete anorexia and vomiting There was some edema of the legs There was no liver or splenic enlargement A few months later he died suddenly, but the cause of death was not ascertained

Unfortunately, satisfactory collections of the stools could not be made, but the amount of urobilin appeared to be very low On one day no urobilin could be detected and on two others amounts of 216 and 1,728 were found

The urine over a period of nine days showed a daily average of 1,292

In the absence of any indication of gross liver disease, the most probable interpretation of the appearance of the large amounts of urobilin in the urine in this case is to be found in damage to the functional efficiency of the liver by excessive amounts of alcohol

In a patient with a huge retroperitoneal sarcoma who was cachectic and feverish, the average urobilin excretion in the urine was 1,545 and 11,893 in the stools A case of carcinoma of the stomach without any metastases in the liver had an amount in the urine corresponding to 794, and 4,576 in the stools

These cases are more difficult to interpret than two others in which there were very large masses of cancerous tissue in the liver, in both of which urobilin was found in great excess in the urine

The patient with cardiac decompensation and swollen liver, whom we mentioned in our first paper, had an average of 2,682 in the urine and 8,619 in the stools

Three cases of effusion of blood into the tissues were examined, but cannot be said to throw any light on the question as to whether or not

urobilin in the urine may have its origin in the breaking down of hemoglobin outside the vessels One was a case of lymphatic leukemia with hemorrhages into the pharynx, gums and orbital cavities There was no urobilin in the urine and only 2,792 in the stools

In a case of caseous tuberculous peritonitis with a large amount of blood in the peritoneal cavity there was an average urinary excretion of 1,264, and in a large pulmonary hemorrhage in a case of compensated mitral stenosis there was an average of 415 in the urine and 15,106 in the stools There are too many other possibilities which might account for the urobilin in the urine in the two cases in which it was present Cases of complete occlusion of the common duct with large hemorrhages are the only ones by which it is possible to decide this point

A disturbance of liver function is, of course, a possibility which is difficult to exclude in almost any case, but it does not seem justifiable at present to attribute every case of excessive excretion of urobilin in the urine without increase in the stools to this cause alone

XI CONCLUSIONS

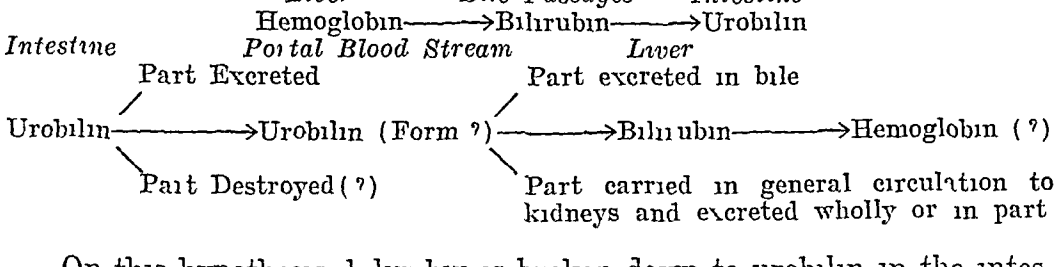
A General

1 The intestinal formation of urobilin from the decomposition of bile present within the bowel is the usual mode of origin In complete closure of the common duct due to carcinoma, for instance, there is usually absence of urobilin from the urine, bile and stools

2 There is evidence that the diseased liver may originate urobilin either directly as a product of its cells, or indirectly from decomposition of bilirubin within the bile passages Thus, patients in whom no bile is reaching the intestine, may nevertheless show urobilin in large amount in the bile, and to some extent even in the urine and stools if the liver itself is diseased or functionally deficient The urobilin in the urine and stools of such cases must be due to excretion from the blood of urobilin absorbed from the liver

The usual sequence in hemoglobin metabolism may be represented as in the accompanying scheme

SCHEMATIC REPRESENTATION OF USUAL SEQUENCE IN HEMOGLOBIN METABOLISM



On this hypothesis, bilirubin is broken down to urobilin in the intestine and the absorbed urobilin is synthesized in the liver to bilirubin and perhaps by a reverse action again to hemoglobin

Under abnormal conditions in the liver, formation of bilirubin from the absorbed urobilin from the intestine may fail, and further there may be a reduction of bilirubin to urobilin in the liver itself, a reversal of the normal process

Under these circumstances, the accumulated urobilin in the liver will be absorbed by the blood-stream and excreted in the urine

3 An increased quantity of urobilin in the stools indicates increased blood destruction

In such cases, urobilin may appear in excessive amount in the urine apart from any obvious disease of the liver, because the quantity of urobilin carried to the liver from the intestine being so much increased, the fraction carried past the liver into the general circulation and so to the kidneys is sufficient to lead to an obvious urobilinuria

4 There is a close association between increase of urobilin in the urine and disturbance of liver function But even if increased blood destruction is excluded by urobilin estimations in the stools, it is not at present possible to regard the amount of urobilin in the urine as an approximate guide to the functional efficiency of the liver The occasional occurrence of large amounts of urobilin in the urine of patients in whom there is no reason to suspect damage to the liver, the great and irregular fluctuations of urobilin excretion in cases of liver disease from hour to hour and from day to day, and the want of knowledge as to the fate of urobilin in the blood and tissues make it necessary to recognize that there may be other factors besides the condition of the liver and the amount of blood destruction which influence the excretion of urobilin in the urine

B Clinical

Before discussing the clinical importance of the phenomena of urobilin elimination, we should like to draw particular attention, (1) to the insufficiency of our present quantitative methods of urobilin estimation especially in the urine, and to state that they undoubtedly will be very difficult to overcome because of the marked instability of the group of substances included under this name, and (2) to the value, until now insufficiently appreciated, of estimations of urobilin in the stools as a guide to the amount of blood destruction in the body

The interpretation of urobilin findings in the urine is interesting but at present somewhat problematical, whereas the determination of an increased intestinal excretion rests on a much firmer basis, and should yield results of diagnostic importance

Since this work was undertaken with the primary object of finding out just how much significance should be attached to the presence of abnormal quantities of urobilin and urobilinogen in the excretions in ordinary clinical work, we shall summarize briefly our conclusions, basing

them on our own experimental studies, clinical observations and a fairly exhaustive study of the literature The difficulty of the problem has been to form a judgment as to how far one is able clinically to make assertions from the data at present obtainable To choose the essential facts from the maze of non-essential detail and from the numerous observations made to substantiate a previously accepted theory has been difficult Nevertheless, we feel that we are within safe limits in the following conclusions as to the significance of the presence of abnormal quantities of urobilin in certain pathological conditions

1 *Hepatic Cirrhosis*—Urobilinuria is of marked value as evidence of a definite pathological change in the enlarged liver of alcoholic persons and occurs almost constantly in the hypertrophic stage of hepatic cirrhosis In our cases, we did not find any increase of urobilin in the stools While in advanced cases, part of the urobilin in the urine may be derived from the formation of urobilin from bilirubin in the liver, it is probable that as a rule it is due to failure on the part of the liver to synthesize to bilirubin, the urobilin brought to it from the intestine

2 *Hepatic Stasis*—Estimations of urobilin in the urine are of value in judging the amount of damage done to the liver parenchyma by chronic passive congestion Hence, a marked increase of it is of ominous prognostic significance in cardiac decompensation

3 *Jaundice*—Urobilinuria is absent or insignificant in cases of obstructive jaundice Its intermittent occurrence points to an incomplete obstruction with concomitant damage to the liver It is present in the icterus of cases of increased destruction of the red blood-cells Careful studies for urobilin should be made in suspected cases of chloroform poisoning, acute yellow atrophy and other similar conditions in which the liver is apt to be damaged

4 *Malaria*—The great increase of urobilin in the stools and the urobilinuria which occurs in severe cases of malaria is of diagnostic importance in obscure febrile conditions The persistence of urobilinuria after malarial attacks, when there is no fever and no increase of urobilin in the stools, can be taken as evidence of some complication such as hepatic cirrhosis or abscess

5 *Anemias*—By means of urobilin estimations in the stools and urine, those forms of anemia associated with an increased blood destruction may be differentiated It is probably in this field that the most valuable clinical results will be obtained The contrast between the very large total urobilin elimination in our cases of pernicious anemia as compared with the small amount found in secondary anemias following hemorrhage or carcinoma was very striking

6. *Pneumonia*—A slight increase of urobilin with additional amounts at the time of resolution does not indicate a severe damage of the liver

parenchyma in this disease It is probably due to a clogging of the liver with the products of resolution and absorbed hemoglobin from the affected lung A study of the curve of this elimination may be of value in certain pneumonias Allowances must always be made for other factors such as constipation, diarrhea and the inability of the kidney to eliminate urobilin readily in the presence of a complicating severe nephritis The early appearance of large quantities of urobilin in the urine in pneumonia, particularly in the presence of jaundice makes the prognosis grave The occurrence of urobilin in the serum of pneumonia patients is of the gravest prognostic significance It is recognized apparently by our present methods only in the presence of a degree of cyanosis markedly unfavorable to the life of the individual

7 *Carcinoma*.—Except in those cases in which there is practically a complete disappearance of urobilin from the excretions, due to an obstruction of the common duct from carcinoma, we have not found its estimation in carcinomatous cases to be of evident clinical value

8 *Infections*.—In infectious processes causing parenchymatous changes in the liver or accompanied by hemolysis, urobilin estimations are of definite value in estimating both of these factors in any given case There is apparently an approximation between the urobilin excretion in the urine and feces and the amount of blood destruction In such infectious processes as amebic colitis, the appearance and constant presence of urobilin may be of marked value in indicating the presence of inflammatory liver conditions, particularly abscess

9 *Scarlet Fever, Measles*.—The majority of measles cases present urobilinuria in excess, but, as might be expected, the amount is less and the duration shorter than in scarlet fever with its more definite damage to the hepatic cells

10 *Decompensation*.—As indicated previously, marked urobilinuria is indicative of pathological change in the liver in the stasis of decompensation This circulatory stasis calls for the use of increasing amounts of hemoglobin within the body and is associated with a greater production and elimination of bilirubin leading to an inability on the part of the overburdened and congested liver to modify the abnormal amounts of urobilin reaching it A return of compensation is often indicated by the disappearance of a qualitative urobilinogen or urobilin test Decompensations in markedly anemic persons are not so apt to be accompanied by increased amounts of urobilin until very profound stasis occurs

11 *Nephritis*.—Severe nephritis of various types, even in the presence of increased production of urobilin, may prevent definite urobilinuria Large amounts of urobilin in the urine may be taken as indirect evidence of a certain degree of efficiency in the renal epithelium

BIBLIOGRAPHY

No attempt has been made to give a complete bibliography, except of the more recent literature The earlier work is quoted by Hildebrandt (Ztschr f klin Med, 1906, lx, 351), by Fischler (Das Urobilin, Diss Heidelberg, 1906), and by Lemaire (Thèse de Paris, 1904-1905, xxvi) Instead of the titles of the papers being given, their bearing on the subject of urobilin has been indicated

Auché The Urobilin Spectrum, Comp rend Soc de biol, 1907, lxiii, 711
A New Method, ibid, p 713
Spectroscopic work on bile, Compt rend, Acad d sci, 1908, cxlvi, 496
Urobilin and Urobilinogen, Compt rend Soc de biol, 1908, lxv, 757
Urobilin in Bile, ibid, 1908, lxv, 758
Method of Making Pure Urobilin, Compt rend Soc de biol, 1909, lxvii, 227
Urobilin in Serum, ibid, 1909, lxvii, 225
Method of Quantitative Estimation, ibid, 1909, lxvii, 229
Audibert Prolonged Catarrhal Icterus, Rev de méd, 1907, xxvii, 544
Austin and Oldway Urobilin in Bile, Boston Med and Surg Journ, 1908, clviii, 763
Baldi Urobilin and Liver, Lo Sperimentale, 1884, liv, 154
Bar Urobilin in Serum, Bull Soc d'obst de Paris, 1903, vi, 149
Baud Urobilin in Cerebrospinal Fluid, Soc de Biol, 1903, lv, 1498
Bargellini Urobilin in Typhoid, Lo Sperimentale, 1892, xlvi, 119
Bauer Ehrlich's Reagent in Urine and Stools, Zentralbl f inn Med, 1905, xxvi, 833
Baumler Liver Cirrhosis and Urobilin, Deutsch med Wchnschr, 1912, xxxviii, 201
Baumstark Estimation of Decomposition Products in Urine and Stools by Ehrlich's Reagent, Munchen med Wchnschr, 1903, li, 722
Beck Formation of Urobilin, Wien klin Wchnschr, 1895, viii, 617
Berggrun and Katz Urobilin and Tuberculous Peritonitis, Wien klin Wchnschr, 1891, iv, 858
Von Bergmann Cerebral Lesions, Volk Sammlung klin Vortr, 190, p 1541
Beyer Trional Poisoning, Deutsche med Wchnschr, 1896, xx, 6
Biffi Urobilin in Blood, Folia haematol, 1907, iv, 533, Bull d Sc med de Bologna, 1907, viii, 309
Bleischroder Urobilin in Post-Mortem Serum, Berl klin Wchnschr, 1909, xlvi, 2, p 2303, cited by Moller
Bourcet Hematoporphorin in Meconium, Soc de Biol, 1910, lxix, 18
Brauer Researches on Liver, Ztschr f physiol Chem, 1903-1904, xl, 182
Braunstein Urobilin in Stomach Contents, Ztschr f klin Med, 1903, l, 159
Brissaud and Bauer Experimental Researches on the Relative Elimination of Bilirubin, Urobilin and Urobilinogen in the Rabbit, Soc de Biol, 1908, lxiv, 809
Brugsch and Yoshimoto Hemoglobin, Bilirubin and Urobilin, Ztsch f exper Path u Therap, 1911, viii, 639
Brugsch and Kawashuma Ibid, 1911, viii, 639
Brugsch and Retzlaff Ibid, 1912, xii, 508
Brugsch Hemoglobin and Bilirubin, Fol haematol, 1910, ii, x, 385
Brugsch Folia haematol, 1911, xii, I T, No 1, 1911, p 23
Cavazza Urobilin in Urine, Il Policlin Sez Medica, 1901, viii, 503
Chaffard Congenital Icterus, Semaine méd, 1907, xxvii, 25
Chalnas Preparation and Quantitative Estimation of Urobilin, Biochem Ztschr, 1909, xx, 401
Chauffard and Rendu Urobilin in Feces, Clinical Value, Press méd, 1907 l, 545
Chase Urobilin and Liver Conditions, Jour Am Med Assn, 1912, xlix, 329
Chiens Theories of Origin of Urobilin, Thése, Toulouse, 1903

Clemens Ehrlich's Reagent in Pathological Urines, Deutsch Archiv f klin Med, 1901, lxxi, 520
Connor and Roper Bilirubin, Urobilin and Urobilinogen, THE ARCHIVES INT MED, 1908 1909, ii, 532
De Jager A Yellow Substance in Urine, Ztschr f physiol Chem, 1910, lxx, 60
Denigès Researches on Urobilin, Soc de Biol, 1897, xlix, 287
Deroide Urobilin Qualitative Test, Soc de Biol, 1898, l, 302
Descomps Methods, Thèse de Paris, 1909 1910, xv
Dick Diagnostic Value of Urobilin in Gynecology, Arch f Gynak, 1884, xxiii, 126
Disqué Chemical, Ztschr f Physiol Chem, 1878-1879, ii, 259
Dor Urobilin in Gasteropods, Soc de Biol, 1902, liv, 54
Doyon, Gautier and Policard Action of Chloroform on Elimination of Urobilin, Soc de Biol, 1908, lxv, 574, ibid, 1909, lxvi, 616
Durand Urobilin, Progrès méd, 1910, xxxviii, 44
Ehrlich The Dimethylamidobenzaldehyd Reaction, Med Wchnschr, 1901, No 15, p 151
Ehrlich and Proscher Ehrlich's Reagent, Ztschr f physiolog Chem, 1901, xxxi, 520
Eichholz Urobilin and Allied Pigments, Jour Physiol, 1893, xiv, 326
Engel and Kiener *Cause of the Brown Color of Urine with Nitric Acid*, Soc de Biol, 1887, iv, 186
Relation of Urobilin to Icterus, ibid, p 225
Falk and Saxl Relation Between Different Functional Liver Tests, Ztschr f klin Med, 1911, lxxiii, 325
Filehne Uselessness of Examination for Urobilin Post-Mortem, Virchows Arch f path Anat, 1890, cxxi, 605
Fischer *Chemical, Urobilin a Mixture of Substances*, Ztschr f physiol Chem, 1911, lxxiii, 204, lxxv, 232
Fischer Simple Spectroscopic Recognition of Hemibilirubin (Qualitative), Munchen med Wchnschr, 1912, lix, 2555
Fischer and Bartholomans Pyrolle Derivatives, Ztschr f phys Chem, 1912, lxxvii, 186
Substituted pyrolles, Ztschr f phys Chem, 1912, lxxvi, 478
Fischer and Meyer-Betz *Method of Preparation of Urobilinogen*, Ztschr f Physiol Chem, 1911, lxxv, 232
Fischer and Meyer Hemibilirubin, ibid, 1912, lxxv, 339
Fischler Urobilin, Diss, Heidelberg, 1906
Fischler Urobilin, Munchen med Wchnschr, 1906, liii, 1784, ibid, 1908, lv, 1421, Deutsche med Wchnschr, 1908, xxxiv, 869, Deutsche Arch f klin Med, 1908, xciii, 426
Fischler and Bardach Eck's Fistula and Urobilin, Ztschr f phys Chem, 1912, lxxviii, *435*
From Urobilin in Cerebrospinal Fluid, Gaz d hôp, 1903, lxxvi, 1257
Fromholdt Urobilin in Rabbits, Ztschr f physiol Chem, 1907, liii, 340
Urobilin and Hydrobilirubin, Ztschr f exper. Path u Therap, 1910, vii, *No 3, p 716*
Urobilin and Reduction in the Intestine, ibid, 1911, ix, No 2, p 268
Fromholdt and Nersessoff Negative Results in Muller's Experiment Except with Fresh Bile, Ztschr f exper Path u Therap, 1912, xii, 400
Fromholdt and Nersessoff Urobilin in Blood, Ztschr f exper Path u Therap, 1912, xii, 404
Garrod and Hopkins Preparation of Urobilin, Jour Physiol, 1896, xx, 112
Percentage Composition, ibid, 1898, xxii, 451
Garrod Yellow Pigment of Urine, Jour Physiol, 1897, xxi, 190
Urinary Pigments, Lancet, London, 1900, ii, 1323
Gautier Origin of Urobilin in Feces, Soc de Biol 1909, lxvii, *205*
Gautier and Monod Urobilin in Urine, ibid, 1909, lxvi, 211

Gautier and Russo Normal Excretion of Urobilin in Rabbit, ibid, 1908, lxiv, 1026
Gerhardt Hydrobilirubin and Relation to Icterus, Diss, Berlin, 1889
Urobilin, Ztschr f klin Med, 1897, xxxii, 303
Pathogenesis of Icterus, Munchen med Wchnschr, 1905, lii, 889
Urobilin, Ztschr f klin Med, 1897, xxxii, 303
Giarré Clinical, Lo Sperimentale, 1895, xlix, 99
Gilbert and Herscher Physiological Stercobilin, Soc de Biol, 1907, lxiii, 452
Pathological Stercobilin, ibid, 1907, lxiii, 597
Formation of Stercobilin, ibid, 1907, lxiii, 802
Bile in Normal Blood, Presse méd, 1906, xiv, 201
Stercobilin, ibid, 1908, xvi, 545
Color of Serum, ibid, 1901, ix, 291
L'ictère hémaphéique, Presse méd, 1902, x, 1239
Acholuric Icterus, ibid, 1903, xi, 541
Renal Origin of Urobilin, ibid, 1902, x, 615
Grigaut Urobilin in Blood ibid, 1909, lxvi, 725
Grigaut and Monod Urobilin in Urine, ibid, lxvi, 211
Grimm Urobilin in Urine, Arch f Path u Anat u Physiol, 1893, cxxxii, 246
Graham Urobilin in Urine After Quinin, Ann Trop Med and Par, 1911, v, 391
Grimbert Urobilin and Urobilinogen, Jour de pharm et chem, 1911, sér 7, iii, 425
Urobilin and Urobilinogen, Soc de Biol, 1911, lxx, 314, 364, ibid, 1897, lvi, 599
Gunther Haematoporphyrinuria, Deutsch Arch f klin Med, 1911, cv, 89
Harley Formation of Urobilin, Brit Med Jour, 1896, ii, 898
Herzfeld Quantitative Estimation of Bilirubin by Spectrophotometer, Ztschr f phys Chem, 1912, lxxvii, 280
Hess and Saxl Rôle of Liver in Blood Destruction, Deutsch Arch f klin Med, 1911, civ, 1
Hess Urobilinogen in Scarlet Fever, Med Klin, 1912, viii, 294
Hildebrandt Urobilin and Icterus, Ztschr f klin Med, 1906, lix, 351
Urobilin, Deutsche med Wchnschr, 1908, No 12, p 489
Significance of Urobilin, Munchen med Wchnschr, 1909, No 14, p 710
Origin of Urobilin, Deutsch med Wchnschr, 1908, lv, 2161
Urobilin in Serum, Munchen med Wchnschr, 1910, lvii, 2574
Urobilin in Pneumonia, Ztschr f klin Med, 1911, lxxiii, 189
Hoppe-Seyler Excretion of Urobilin in Diseases, Virchows Arch f path Anat, 1891, cxxiv, 30
Formation of Blood Pigments, Ber d Deutsch chem Gesellsch, 1874, vii, 1065
Huber Reason and Meaning of Urobilin, Charité Ann, 1906, xlix, 52
Bilirubin and Urobilin, Med Klinik, 1910, vi, 56
Huppert Analyse des Harns 1898, p 533
Jaffé Urobilin, Virchow's Arch f path Anat, 1869, xlvii, 405
Jonas Urobilinogen in Heart Disease, Zentralbl f die ges inn Med, 1912, i, 454
De Jonge (quoted by Simpson) Great Increase of Urobilin in Stools in Malaria
Justi Urobilin in Tropical Practice, Zentralbl f die ges inn Med, 1912, ii, 694
Hayem Diagnostic Value of Urobilin, Gaz d hôp, 1889, lxii, No 144
Hopkins Urobilin, Guy's Hosp Rep, 1893, l, 351
Katz Clinical, Wien med Wchnschr, 1891, xli, 1194, 1295, 1326
Kayser Handbuch d Spektroskopie, 1908
Kimura Researches on Urobilin in Bile, Deutsch Arch f klin Med, 1904, lxxix, 274

Von Koziczkowski Clinical Worth of Ehrlich's Reagent, Berl klin Wchnschr, 1902, No 44, p 1029
Kunkel Urinary Pigments, Virchows Arch f path Anat, 1880, lxxix, 455
Kuster Bile Pigments, Ztschr f physiol Chem, 1906, xlix, 391
Bilirverdin, ibid, 1909, lix, 63
Hematin, ibid, 1909, lxvi, 164
Blood Pigments, ibid, 1910, lxvi, 165
Labbé and Carée Urobilin in Stools and Urine in Obstructive Jaundice, Soc de Biol, 1910, lxx, 793
Ladage Injections of Urobilin, Thesis Leiden, 1899
Lehndorff Bilirubin and Urobilin in Blood Serum and Serous Fluids, Prag med Wchnschr, 1912, xxxvii, 495
Lemaire Urobilin, Thèse de Paris, 1904 1905, xxvi
Urohematin, Presse méd, 1904, xii, 644
Lepehne Blood-Counts in Cats with Closed Common Ducts, Thesis, Konigsburg, 1910
Leppmann and Husschmidt Urobilinogen and Inclusion Bodies in Scarlet Fever, Zentralbl f inn Med, 1913, xxxii, 369
Lesage Urobilin in Urine after Poisoning Dogs with Beta Naphtholin, Soc de Biol, 1904, lvi, 1026
Lesieur, Monod and Morel Urobilin, ibid, 1908, lxiv 343
Lesieur and Méret Is Urobilin Normally Present? Presse méd, 1908, xvi, 501
Leyko and Marchlewski Hæmopyrole, Biochem Ztschr, 1908, x, 437, ibid, 1909, xxii, 464
Levy, Magnus Formation of Urobilin in Autolysis of Liver, Beitr z chem Physiol u Pathol (Hofmeister's), 1902, ii, 261
Macfadyen, Nencki and Sieber Chemical Processes in Intestines, Arch f exper Path u Pharmakol, 1891, xxviii, 311
Marchlewski Blood Pigments, Ztschr f physiol Chem, 1909, lxi 276
Meinel Origin of Urobilin, Zentralbl f inn Med, 1903, xxiv, 321
Formation of Urobilin in Stomach, ibid, xxiv, 441
Minkowski Pathogenesis of Icterus, Ztschr f klin Med, 1904, lv, 34
Moller Origin of Urobilin, Berl klin Wchnschr, 1909, xlv, 1639, ibid, 1909, xlvi, 2303
Monges Origin of Fecal Urobilin, Soc de Biol, 1908, lxvii, 609
Mongour Urobilin in Serum, Sem méd, 1908, xxviii, 456
Mongour and Zellmeyer Urobilin in Serum (Sylabba's Method), X Congrès franç de méd, 1908, p 211, Bull méd, 1908, xx, 816
Mongour and Chevrier Uselessness of Grimbert's Method, Soc de Biol, 1911, lxx, 664
Monod Origin of Urobilin, Arch de l'app dig et de la nut, 1909, iii, No 5 p 366
Moracewski Quantitative Estimate of Urobilin in Stools by Spectroscope, Arch f Verdauungskrankh, 1908, xiv, 378
Morel and Monod Method, Soc de Biol, 1908, lxiv, 205
Mosse Polycythemia with Urobilin in Serum, Deutsch med Wchnschr, 1907, xxxiii, 2175
Muller, F Icterus, Ztschr f klin Med, 1887, xii, 45, Ztschr f Biol, 1884, xx, 327 Proceedings "Innere Medizin," 11th Congress, 1892, p 118
Muir and M'Nee Anemia Produced by Lytic Serums, Journ Path and Bacteriol 1912 xvi, 410
Munzer and Bloch Clinical, Arch f Verdauungskrankh, 1911, xvii, 260
Mya Method, Ann des Mal Gén Urin, 1891, ix, 682
Nencki and Zaleski Experimental and Chemical, Ber d deutsch chem Gesellsch, 1901, xxxiv, 997
Nencki and Sieber Production of Urobilin from Hematoporphyrin Arch f exper Path, 1888 xxiv, 430
Neubauer Ehrlich's Reagent, Munchen med Wchnschr, 1903, 1 1846

Neubauer Sitzungsberichte d Gessel f Morph u Physiologie, Munchen, 1903, ii, 32
Neubauer Injection of Hematoporphyrin, Arch f exper Path u Therap, 1900, xliii, 456
Pel Relation of Spleen to Bile Formation, Deutsch Arch f klin Med, 1912, cvi, 250
Perl Urobilinuria, Inaug Diss, Berlin, 1912
Pick Toluylendiamin-Icterus, Wien klin Wchnschr, 1892, xlii, 307
Blood-Pigments, Liebig's Ann, 1909, cccl\xvi, 237
Constitution of Blood Pigments, ibid, 1910, cccl\xxvii, 314
Piloty and Meirzbacher Haematopyrollacid, Ber d d chem Gesellsch, 1909, xlii, 3253
Splitting of Hematoporphyrin, ibid, p 3258
Piloty and Quitman Constitution of Hemopyrolle, ibid, p 4663
Proscher Ehrlich's Reagent, Deutsch med Wchnschr, 1903, xxix, 927
Querrolo and Benvenuti Icterus, Policlinico, 1900, vii, 374
Rach and Reuss Urobilin in Measles, Ztschr f Kinderheilk, 1912, ii, 460
Rank Urobilin, Munchen med Wchnschr, 1902, xlix, 1620
Roth and Hersfeld Urobilin in Blood, Deutsch med Wchnschr, 1911, xlvi, 2129
Renvers Urobilin from Infarct of Lung, Deutsch med Wchnschr, 1892, xviii, 254
Rhode Ehrlich's Reagent, Ztschr f physiol Chem, 1905, xliv, 161
Rocher Urobilin in Retention Icterus, Thèse de Paris, 1911, xxxix, No 406
Rubin Urobilin in Typhoid, Munchen med Wchnschr, 1907, No 11, p 507
v Rzentkowski Bile, Biochem Ztschr, 1909, xvi, 146
Saillet Urobilin in Normal Urine, Rev de Méd, 1897, xvii, 109
Salkowski Virchow's Arch f path Anat, 1887, cix, 358
Recognition of Urobilin and Bilirubin in Feces, Berl klin Wchnschr, 1897, xxxiv, 353
Scheel Bilirubin in Blood, Ztschr f klin Med, 1912, lxxiv, 13
Schlesinger Qualitative Test for Urobilin, Deutsch med Wchnschr, 1903, xxix, 561
Schmidt Urobilin, Arch f exper Path u Pharmakol, 1906-1907, lvi, 130
Schmidt, Ad Hydrobilirubin, Verhandl der Kong f inn Med, 1895, xiii, 320
Ehrlich's Solution for Quantitative Estimation of Indol, Munchen med Wchnschr, 1903, l, 721
Simon Discussion on Ehrlich's Reagent, Am Jour Med Sc, 1903, cxxvi, 471
Simpson Urobilin in Stools, Quantitative Relation to Hemoglobin, Biochem Jour, 1911, v, 378
Hemoglobin Metabolism in Malaria, Ann Trop Med and Parasitol, 1910, iv, 313
Case of Blackwater Fever (Ross, Thompson and Simpson), Ann Trop Med and Parasitol, 1910, iv, 307
Hemoglobin Metabolism in Malaria, Proc Roy Soc, 1910, lxxxiii, H B, p 562, 174
Soucek Renal Origin of Urobilin, abstr Zentralbl f inn Med, 1906 xxvii, 684
von Starch Urobilin Content in Anemia of Children, Jahrb f Kinderheilk, F ii, Erganz, 1900, p 421, lii, F ii, p 421
Staunig Coincidence of Urobilin and Albumin in Cardiac Disease, Zentralbl f d ges Inn Med, 1912, i 509
Steensma Urobilin in Stools, Nederl Tijdschr v Geneesk, 1907, i, 273
Qualitative Test for Bilirubin, Biochem Ztschr, 1908, viii, 209
Stern Function Tests of Liver, Deutsch med Wchnschr, 1905, xxxi, 1104
Stich Urobilin in Exudates, Munchen med Wchnschr, 1901, xlviii, 1751
Strauss Methods, Urobilin in Serum, Munchen med Wchnschr, 1908, lv, 2537

Surveyor Clinical, Biochem Jour, 1908, iii, 439
Syllaba Urobilin in Serum, Fol haematol, 1904, i, 636
Urobilin in Serum, Fol haematol, bd I, Ref 33, p 283
Urobilin in Serum, Deutsch med Wchnschr, 1912, xxxviii, 900
Tarchanoff Effect of Hemoglobin on Bile Secretion, Arch f d ges Physiol, 1874, ix, 53
Tefig and Ibrahim Urobilin in Urine, Ztschr f Urol, 1909, iii, 703
Thomas Urobilinogen, Diss, Freiburg, 1907
Troisier Hemolysis and Urobilin, Soc de Biol, 1909, lxvi, 739
Urobilin in Blood in Icterus, ibid, lxvii, 46
Parenteral Origin of Urobilin from Hemoglobin, Thése de Paris, 1910, No 429
Triboulet Reduction of Bilirubin to Urobilinogen by Lymphoid Tissue, Soc de Biol, 1910, lxix, 345
Tsuchiya Parenteral Origin of Urobilin, Arch f exper Path u Therap, 1910, vii, 352
Ury Ehrlich's Reaction in Stools, Zentralbl f inn Med, 1906, xxvii, 40
Viglezio Spec Visibility of Urobilin, Lo Sperimentale, 1891, xlv, 225 (Quoted by Simpson)
Ville Action of Palladium Hydrogen on Bile, Soc de Biol, 1910, lxix, 419
Weil, Moul and Policard Two Pigments in Extracts of Stools of Infants, Soc de Biol, 1911, lxx, 581
Wilbur and Addis Urobilin, Jour Am Med Assn, 1912, lix, 929
Zaleski Urobilin not Identical with an Oxidation Product of Haemopyrolle, cit in Mal's Jahresber de Tierchem, 1905, xxxv, 406, 1906, xxxvi, 455
Zoja Hemoglobin and Urobilin, Arch Folia Haematol, 1910, x, 225
Zoja Hemoglobin Decomposition and Urobilinogen, Folia haematol, 1911, xii, 1

THE EFFECT OF HEAT ON BLOOD-PRESSURE *

L H NEWBURGH, M D,† AND C H LAWRENCE, M D
BOSTON

In most infectious disease there is a progressive fall in blood-pressure during the course of the infection This fall has been attributed to a weakness or insufficiency of the circulatory apparatus In 1899 Romberg, Passler, Bruhns and Muller[1] investigated the cause of this presumed weakness by means of animal experiments They reached the conclusion that the myocardium was not at fault but that the toxin of the infection caused a grave injury to the vasomotor center in the medulla, and that the low pressure was due entirely to an abeyance of the function of this center Since that time it has been generally held by clinicians that the height of the blood-pressure in the infectious diseases was a measure of the tone of the vasomotor center, that low readings were evidence of the failure of the center and were an absolute indication for therapeutic procedures which stimulated the center

During the past year one of us (L H. N) has been studying, at the Massachusetts General Hospital, the therapy of the circulatory disturbances in the acute infections based on these principles The results, on the whole, have been such that it seemed desirable to reinvestigate this phase of the problem

Increased body temperature is a symptom of all infections Romberg and Passler[1] failed to eliminate the effect on the blood-pressure of this constant factor It therefore remains undecided whether the hypotension observed by them was due to toxins, fever, or both

In the experiments on which this paper is based the effect of increased body temperature, in the absence of infection, was studied in order that its importance as a factor in causing the lowered blood-pressure of infection might be determined

Hallıburton in 1901[2] pointed out that all tissues contain a protein which coagulates between 45 and 50 C (113 and 122 F), that during

* From the Department of Medicine and the Laboratory of Physiology, Harvard Medical School

* Submitted for publication Oct 6, 1913

† Working under the Dalton Scholarship, Massachusetts General Hospital

1 Romberg, Passler, Bruhns and Muller Experimentelle Untersuchungen uber die allgemeine Pathologie der Kreislaufstorung bei acuten Infectionskrankheiten, Arch f klin Med , 1899, lxiv, 652

2 Hallıburton, W D Chemical Side of Nervous Activity, Croonian Lectures, London, 1901, p 42

heat rigor the first shortening of mammalian muscle occurs at 47 C (116 6 F). and that it loses its irritability—that is, is physiologically dead —at that point Marinesco[3] has shown that a temperature at 45 C (113 F) kills animals in from one to two hours, and 43 C (109 4 F) in a longer time, and that the occurrence of death is coincident with the breakdown of the nerve-cells Hallıburton[2] found that saline extracts of brains kept at a temperature of 44 C (11 2 F) show a precipitation of globulin after two hours When the temperature is 42 C (107 6 F), the precipitation occurs in from three to four hours A temperature of from 40 to 41 C (from 104 to 105 8 F) causes no coagulation after eight hours Thus we see that degrees of hyperthermia occasionally seen in infections cause morphological changes in nervous tissue, and that lower temperatures produce effects identical with higher ones if the former obtain over longer periods of time

One of the factors which is necessary to maintain an efficient blood-pressure is a constant constriction of the arterial tree To maintain this constriction there must exist not only an active nervous system, but also smooth muscle capable of responding normally to stimuli The relaxing effect of heat on smooth muscle is well known and must be taken into account when considering the effect of heat on blood-pressure It has recently been noticed by clinical observers[4] that the blood-pressure is lowered during the application of heat in the form of hot baths or similar maneuvers in nephritis, etc It is not known whether the body temperature was raised as a result of this form of treatment

Our experimental procedure was as follows Rabbits and cats were anesthetized with urethane (ethyl carbamate) employed in a dosage of 2 gm per kilogram, given by stomach-tube Occasionally an additional gram of urethane was required in order to obtain complete anesthesia Blood-pressure was written from the carotid artery by means of a mercury manometer The animals were placed in a copper tank which contained sufficient water at body temperature to cover the animal except its head The water was heated by means of an insulated electric resistance-coil in the bottom of the tank The temperature of the water and the animal's rectal temperature were recorded Fourteen experiments were performed

In the first group of experiments the temperature of the animals was rapidly raised The first effect of the heat was a progressive rise in blood-pressure At a temperature of between 108 and 110 F, varying

3 Marinesco Quoted by Hallıburton

4 Lawrence, C H The Effect of Pressure Lowering Drugs and Therapeutic Measures on the Systolic and Diastolic Pressures in Man, THE ARCHIVES INT MED, 1912, IX, 409

Fig 1, Experiment 10—The upper line is the carotid blood pressure, the lower line is 50 mm Hg pressure *A* shows the pressure at the beginning of the experiment The record of the next fifty minutes is omitted *B* is the tracing obtained from the fiftieth to the sixtieth minute The cat's temperature was rising from 103 7 to 104 5 F The temperature of the water was 107 6 F The respiration was too rapid to count accurately—about 400 per minute—shallow, panting, but still regular At *C* the wide variations occurring toward the end of the second hour—the one hundred and fifth to the one hundred and twentieth minute—are shown The cat's temperature was from 108 to 108 5 F The temperature of the water was 109 F The respiration was more than 400 per minute, shallow and irregular, but the mucous membranes were pink *D* represents the last forty five minutes of the experiment—from the one hundred and thirty-fifth to the one hundred and eightieth minute It showed a steady fall in pressure broken only by the response to asphyxia at *X* The small amplitude of the pulse is noteworthy During this period the cat's temperature rose slowly from 108 9 to 110 F The temperature of the water was 109 F During the third hour the respiratory rate has been slowly decreasing and had fallen to 120 per minute just before the effect of asphyxia was tried It was constantly irregular

with the individual, the pressure began to fall and continued to do so until the end of the experiment Experiment 10 is cited in detail as an example of this group

EXPERIMENT 10.—July 28, 1913, a female cat weighing 4 kg was given 8 gm of urethane by stomach tube at 10 a m A cannula was inserted into the left carotid artery, and a cannula into the trachea A thermometer was inserted as high as possible into the rectum The animal was transferred to the tank containing water at a temperature of 99 5 F at 10 45 a m A continuous record of the blood-pressure was written Table 1 shows the temperature of the water, the rectal temperature of the cat, the blood pressure level and the rate and character of the respiration, marked off in approximately five minute periods throughout the experiment

TABLE 1.—RECORD OF EXPERIMENT 10

Time, A M	Temp Water, F	Temp Cat, F	Blood-Pressure mm Hg	Respiration
10 55-11 00	99 7	100 0	155	66
11 00 11 03	100 4	100 2	150	94
11 09-11 15	103 0	101 0	150	160
11 15-11 20	104 0	101 0	144	160
11 20 11 25	105 8	101 9	150	260, shallow, panting
11 25 11 30	107 6	102 0	155	400, regular, panting
11 30 11 35	107 6	103 0	162	400, regular, panting
11 35 11 40	107 6	103 7	174	400, regular, panting
11 40-11 45	107 6	104 5	174	400, regular, panting
11 45 11 52	107 6	104 5	*	400, regular, panting
11 52 12 00 P M		106 4	126	400, irregular
12 05-12 25	107 6	†		
12 25-12 30	108 6	107 5	110 136	400, irregular
12 30 12 35	109 0	107 5	88 142	Too rapid to count
12 35-12 40	109 0	108 0	90 148	Too rapid to count
12 40 12 45	109 4	108 2	70 140	Too rapid to count
12 45 12 50	109 4	108 2	100 120	Too rapid to count
12 50 12 55	109 4	108 2	88 112	Very irregular
12 55- 1 00	109 4	108 5	110	Very irregular
1 00 1 05	109 4	108 9	124	Slower
1 05- 1 10	109 4	109 1	117	Slower
1 10 1 15	109 4	109 6	106	Slower
1 15 1 20	109 4	110 0	96	Slower
1 20- 1 25	109 0	110 0	86	120
1 27	109 0	110 0	84 154	Asphyxia reaction
1 30 1 35	109 0	110 0	70 100	66, deep, regular
1 35- 1 40	109 0	110 0	67	52 36, deep, cyanosis

* Clot removed
† Clot Cannula in right carotid

The animal's temperature was raised from 100 to 104 5 F in the course of the first hour The pressure during this period rose from 144 to 174 mm Hg During the second hour the temperature rose from 104 5 to 108 2 F The pressure showed wide variations with a general downward tendency The heart had become markedly irregular and the steep falls in pressure are evidence of this condition Respiration became too rapid to count—more than 400 per minute—irregular and shallow During the third hour the temperature rose from 108 2 to 110 F The pulse had become very small but more regular There was a

progressive fall of blood-pressure to 64 mm Hg The respiratory rate slowly decreased but its character remained the same At X (Chart 1) a rubber tube 1½ feet long was attached to the tracheal cannula As is well known, asphyxia is one of the most powerful stimulants to the vasomotor center The pressure rose from 84 to 154 mm Hg — a response of 70 mm The respirations became deep, regular and slow, falling to thirty-six per minute

It may well be questioned whether the fall of pressure was not a result of great interference with the function of living tissue brought about by the extreme heat In favor of this view we have the great cardiac irregularity and the very rapid, irregular respiration, which then becomes slower with increasing heat On the other hand, the asphyxial rise in pressure indicates that the vasomotor reflex was still intact But since it was primarily our object to investigate the effect of degrees of heat which are the rule in infections, we performed further experiments in which the heat was not allowed to become excessive Experiments 7 and 11 are cited as examples of this group



Fig 2, Experiment 7 —The upper line is the carotid blood pressure The lower line is 50 mm Hg pressure *A* is the pressure at the beginning of the experiment The cat's rectal temperature was 100 F At *B* the pressure is shown forty-five minutes from the beginning of the experiment The cat's rectal temperature was 104 5 F , that of the water 105 8 F Respiration was 260 per minute At *C* the pressure one hour, and one hour and ten minutes, from the beginning of the experiment is shown The cat's temperature was from 104 5 to 105 3 F , the temperature of the water between 105 8 and 106 8 F The respiration had fallen progressively from the beginning of the experiment and was now between 176 and 208 per minute The pressure had fallen 92 mm during the previous twenty-five minutes and was 62 mm lower than at the beginning of the experiment The cat's temperature remained between 105 5 and 106 F for the next hour and twenty minutes, at the end of which time the respiration, which had become steadily slower, suddenly ceased The pressure fell slowly during this period from 96 mm to 65 mm *D* represents the last ten minutes of the experiment

Experiment 7 —July 19, 1913, a male cat, weighing 4 2 kg was given 8 gm of urethane by stomach-tube at 9 20 a m A cannula was inserted into the carotid artery and a thermometer inserted as high as possible into the rectum At 10 15 a m the animal was placed in the tank containing water at a temperature of 99 5 F For the next forty minutes the water was rapidly heated until

it reached 105 8 F During the rest of the experiment it was kept between 105 and 106 8 F Table 2 shows the temperature of the water, the rectal temperature of the cat, the blood pressure level and the rate and character of the respirations throughout the experiment The time notations in the first column represent periods when the drum was revolving

TABLE 2—RECORD OF EXPERIMENT 7

Time, A M	Temp Water, F	Temp Cat, F	Blood-Pressure mm Hg	Respiration
10 17 10 20	99 5	100 0	158	280
10 35 10 38	104 0	102 0	176	260
10 58 11 01	105 8	104 5	188	260
11 15 11 18	105 8	104 5	122	208, shallow, irregular
11 28 11 31	106 8	105 3	96	176, shallow, irregular
11 35 11 40	106 2	105 5	90	176, shallow, irregular
11 40 11 45	106 2	105 5	88	176, shallow, irregular
11 45 11 50	105 8	105 5	84	144, shallow, regular
11 50 11 55	105 8	105 5	84	144, shallow, regular
P M				
12 00 12 05	106 1	105 5	74	144, shallow, regular
12 05-12 10	106 1	105 5	74	144, shallow, regular
12 10 12 15	106 1	105 5	72	144, shallow, regular
12 15-12 20	106 1	105 5	72	144, shallow, regular
12 20 12 30	*			
12 30 12 35	106 7	105 5	65	144, shallow, regular
12 35 12 40	106 7	106 0	65	111, shallow, regular
12 40 12 45	105 8	106 0	65	100 shallow, regular
12 46	105 8	106 0	65 0	64, shallow, regular
12 48	105 8	106 0		Ceased
12 50	†			

* Clot removed
† Heart stopped

EXPERIMENT 11—July 31, 1913, a male cat weighing 4 kg was given 8 gm of urethane at 9 15 a m by stomach tube and 1 gm at 10 15 a m A cannula was inserted into the left carotid artery The left cervical vagus trunk was dissected free and cut A thermometer was inserted high into the rectum and the animal placed in the water at a temperature of 99 5 F The central end of the vagus was then stimulated with an induction current of increasing strength, until something greater than the threshold value had been reached This strength of current was used throughout the experiment The water was now rapidly heated to 104 F and kept at this temperature for the next eight and one half hours The blood pressure was written approximately once an hour during this period After the drum had been revolving for several minutes the central end of the vagus was stimulated with the induced current The nerve was then carefully replaced between the tissues to be kept fresh Several times the nasal mucous membrane was stimulated

The group of experiments of which 7 and 11 are types show a well-marked fall in blood-pressure due to a degree of hyperthermia not exceeding that encountered in infections In all cases the fall occurred before any organic changes due to hyperthermia could be expected to

occur As is seen in Experiment 12, which was performed as a control, no such fall occurred when the animal, kept at a normal temperature, was subjected to all other conditions of the experiment



Fig 3, Experiment 11 —The top line is the carotid blood pressure The next line is 0 mm Hg pressure Portions of the record from the beginning of the experiment, three hours later, and eight and one-half hours later, are shown, 1, *A*, is the initial level of blood pressure, 136 mm , 2, *A*, is the response to vagus stimulation when the pressure is 136, the pressure fell 40 mm , 1, *B*, is the blood pressure level, 114, after three hours, 2, *B*, is the response to vagus stimulation when the pressure is 114, the pressure fell 9 mm , 3, *B*, is the response to vagus stimulation when the pressure is 128, the pressure fell 32 mm , 1, *C*, is the blood pressure level, 72, after eight and one-half hours, 2, *C*, is the response to vagus stimulation when the pressure is 70, the pressure fell 7 mm , 3, *C*, is the response to vagus stimulation when the pressure is 80, the pressure fell 17 mm , 4, *C*, is the response when the pressure is 84, the pressure fell 29 mm

TABLE 3 —Record of Experiment 11

Time, A M	Temp Water, F	Temp Cat, F	Resp	B -P mm Hg	Response to Stimulation
11 20 P 11 33 M	98 6	99 5	48	136	Vagus 10 sec 136— 96 = 40
12 25 12 35	104 0	102 5	214	114	Vagus 1 sec 113—110 = 3
					Vagus 3 sec 116—107 = 9
					Vagus 10 sec 110— 99 = 11
					Vagus 10 sec 128— 96 = 32
1 25 1 35	104 0	103 2	150	118	Vagus 10 sec 119—115 = 4
					Vagus 10 sec 114— 99 = 15
					Vagus 10 sec 130—112 = 18
					Vagus 10 sec 130—103 = 27
2 25 2 35	104 0	103 0	150	114	Vagus 10 sec 114—105 = 9
					Vagus 10 sec 128— 96 = 32
3 25 3 30	104 0	103 2	106	104	Vagus 10 sec 105— 97 = 7
4 35- 4 40	104 0	103 2	106	100	Vagus 10 sec 98— 85 = 13
5 35 5 40	103 6	103 2	106	87	Vagus 10 sec 88— 74 = 14
					Nose 10 sec 88—109 = 21
6 40 6 45	103 6	103 2	100	76	Vagus 10 sec 78— 62 = 16
					Nose 10 sec ? ?
7 40 7 53	104	103 2	100	72	Vagus 10 sec 70— 63 = 7
					Vagus 10 sec 79— 62 = 17
					Vagus 10 sec 84— 64 = 20

EXPERIMENT 12.—Aug. 6, 1913, a male cat weighing 4.2 kg. was given 8 gm. of urethane by stomach tube at 9 a. m., 1 gm. at 9:30 a. m., and 1 gm. at 9:55 a. m. A cannula was inserted into the left carotid artery, and a thermometer high into the rectum. The cat was placed in a tank containing water at 100.4 F. at 10:15 a. m. The cat's temperature fell from 100.4 F. at 9:30 a. m. to 98 F. at 10:15 a. m.

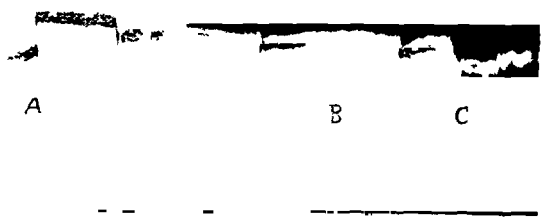


Fig. 4, Experiment 12.—The upper line is the carotid blood pressure, the lower line is 50 mm. Hg pressure, *A* is the initial pressure, *B* the pressure after one hour, and *C* the pressure after two hours. This tracing shows the practical absence of change in the blood pressure level when the animal is kept in water at body temperature.

TABLE 4.—RECORD OF EXPERIMENT 12

Time, A. M.	Temp. Water, F.	Temp. Cat, F.	Blood-Pressure mm. Hg	Respiration
10:20-10:25	100.4	98.6	136	36
10:30-10:32	100.4	98.6	150	36
11:00-11:05	100.4	100.4	144	40
11:30-11:35	98.6	99.5	144	40
12:20-12:25	100.4	100.0	136	40

It appears, therefore, that the fall in pressure was entirely due to the effect of heat on the organism.

DISCUSSION

Our chief interest centers about the fall in pressure which results from degrees of heat of not uncommon clinical occurrence. We know from daily observation that such amounts of hyperthermia do not cause serious organic damage. The hypotension of such conditions, therefore, must be evidence of a functional disturbance.

Since blood-pressure is determined by the energy of the heart as measured by the output per minute against resistance, and on the other hand by peripheral resistance, it is evident that the low pressure might be due to a lessened output from the heart, or to a diminished peripheral resistance, or to both; and the lessened peripheral resistance in turn might result from relaxation of the smooth muscles of the arterioles due

to the heat, or to fatigue of the nerve-cells in control of the vascular mechanism The factors affecting peripheral resistance may be considered first

It has long been held by clinicians that heat has a marked relaxing effect on smooth muscles, but they have consciously or unconsciously thought of this effect in relation to muscle which is undergoing abnormal tonic or clonic contractions We know of no clinical evidence which may be presented to show that heat has the same effect on smooth muscle in a state of normal tonicity DeZilwa[5] and Stewart[6] have shown experimentally that there is a gradual relaxation of the tone of smooth muscle as the temperature is raised from 10 to 40 C (from 50 to 104 F), but that temperatures over 40 C cause a shortening again In their experiments the amount of change occurring between 38 5 and 40 C (101 3 and 104 F) is very small If we seek to explain our results as examples of the decrease of tonicity of smooth muscle in response to heat, we should expect to find that the pressure fell as the temperature rose to 40 C (104 F), and that the pressure rose thereafter with increasing temperature Examination of our tables will show that just the opposite occurred Therefore, we cannot logically attribute the hypotension to this factor

In considering the other possible factor that might diminish peripheral resistance, we have to take into account the conditions of activities of nerve-cells It is highly characteristic of nervous activity that periodic rest is needed to restore normal function The observations of Hodge[7] showed that in nerve-cells definite physiological changes develop during the day and are recovered from during the rest at night "Continuous loss of sleep is far more rapidly fatal than starvation, and the final changes, especially in the nervous system, are very marked" (Donaldson[8]) Howell has shown that sleep is accompanied by a diminution of vascular tone, that is, a lessened activity of the vasomotor center Since, therefore, there is this alternation of fatigue and recovery through long periods during the twenty-four hours, and since the absence of the resting period is known to be harmful to the nerve-cells, the necessity for repair in these cells is obvious

Studies of the effect of temperature on chemical processes have shown that there is approximately an increase of 10 per cent in the speed of reaction with every increase of 1 degree centigrade The metabolic processes occurring in nerve-cells are chemical in character, and a number of observations are on record to show how markedly the speed

5 DeZilwa Quoted by Grutzner, Ergebn d Physiol, 1904, II, 12

6 Stewart, C C The Cat's Bladder, Am Jour Physiol, 1900, IV, 185

7 Hodge Quoted by Donaldson, The Growth of the Brain, London, 1895, p 317

8 Donaldson, H H The Growth of the Brain, London, p 309

of nervous activity is augmented by increased temperature Thus Helmholtz[9] found the velocity of the nerve impulse in the frog at 0 C (32 F) to be 2 7 meters per second, at 20 C (68 F) 27 meters per second Von Miram[9] found the rate for the frog 0 24 meters per second at 20 C, and 65 meters at 35 C (95 F)

Since the evidence from activity and rest indicates that during the active period the catabolic changes are more rapid than the anabolic, it is highly probable that similarly increased heat has a greater effect on the catabolism of the neurons than on the anabolism The increased speed of the catabolic processes which heat would induce might, therefore, result in increased tone of the vasomotor center in the early stages, and that period might be followed by a later period of diminished tone because of approaching exhaustion of the nerve-cells

W T Porter[10] and his associates have brought forward evidence which indicates that the tone of the vasomotor center is not lowered by prolonged peripheral stimulation Such stimulation, however, becomes widely dispersed through the nervous system and only in part affects the vasomotor center Heat, one the other hand, would have as much effect on one cell as on another, and the fact that increased heat is accompanied by diminution of the tonic innervation of skeletal muscle indicates the possibility that a similar condition prevails in the vasomotor center

In two of our experiments we tested the reflex response of the vasomotor mechanism after we had obtained large falls in blood-pressure In every case the normal response occurred, but this evidence does not permit us to draw conclusions as to the tonus of the vasomotor cells, for Porter[11] has shown that the condition of the vasomotor center does not determine the presence or absence of the vasomotor reflex, and that consequently testing the reflex gives no information about the state of tonus of the center

There still remains to be considered the effect of the heart rate on the lessened blood-pressure observed during continued hyperthermia Experiments[12] have shown that the faster the heart beats the shorter the diastole, but within wide limits the abbreviation of the diastole occurs only in that part of it which has been designated diastasis, that is, the period of repose, and does not affect the period of relaxation or filling If the heart is supplied with blood, a more rapid beat within the period

9 Helmholtz and Von Miram Quoted by Snyder, Am Jour Physiol, 1908, xxii, 179

10 Porter, W T, Marks, H K, and Swift, J B The Relation of Afferent Impulses to Fatigue of the Vasomotor Center, Am Jour Physiol, 1907, xx, 444

11 Porter, W T Relation of Afferent Impulses to the Vasomotor Center, Am Jour Physiol 1910, xxvii, 276

12 Henderson, Y A Principle Underlying the Normal Variations in the Volume of the Blood Stream, Etc, Am Jour Physiol, 1909 xxiii, 353

of diastasis results in a greater output Only when the beat becomes so rapid as to encroach on the period of filling does the output fail to vary with the rate of beat The observations, reported in this paper, of increased blood-pressure in the early stages of hyperthermia and lessened blood-pressure after the temperature rose higher, are quite in harmony with the greater output from the heart that would result so long as the rapid beat encroached only on the period of diastasis, and with the lessened output that would result as the still more rapid beat would cause an encroachment on the period of relaxation or filling

This review of evidence indicates that the heat probably does not cause sufficient local relaxation of smooth muscle in the arterioles to account for the low pressure As between a central effect of the heat on the tonus of the vasomotor center and a peripheral effect on the heart, our observations thus far do not permit us to pass judgment Quite conceivably both factors are involved in the production of effects which we have here reported, and as the foregoing discussion has shown, heat would operate in the same direction with both these factors to cause first a rise and later a fall of arterial pressure For the present, therefore, we must leave open the explanation of our results and merely report the facts of observation

CONCLUSIONS

1 In lower animals degrees of hyperthermia not greater than those encountered in infections are sufficient to cause marked hypotension

2 The increased body temperature of infection is a potent factor in the production of the lowered blood-pressure which occurs in such conditions

3 The hyperthermia may be the entire cause of such hypotension

We are grateful to Dr W B Cannon for having suggested the possible effect of heat on blood-pressure to us and for having aided us with many of the technical difficulties

329 Beacon Street—374 Marlborough Street

PAROXYSMAL AURICULAR FIBRILLATION*

G CANBY ROBINSON, M D
ST LOUIS, MO

The abnormalities of the cardiac mechanism which are responsible for various forms of cardiac arhythmia and disturbances in cardiac rate are now well understood, and have been especially well demonstrated by means of the string galvanometer The analysis of disturbances of the cardiac mechanism, although of ever increasing diagnostic and therapeutic importance, is a field for original investigation which is fast losing its fertility On the other hand, large problems lie before us in determining the essential causes of these cardiac disturbances The progress of the treatment of cardiac disorders will be accelerated by the discovery of the now unknown changes, either within the heart or outside of it, which are responsible for these abnormalities of the cardiac mechanism This is especially true of the large group of disorders characterized by disturbances in the generation of stimuli of the heart beat These disturbances may predominate either in the auricles or in the ventricles, and may result in the occurrence of premature ectopic beats (extrasystoles), tachycardia of ectopic origin, the so-called auricular flutter or auricular fibrillation These disturbances may occur in hearts already the site of anatomical lesions, but they may also occur in hearts which give no signs of such lesions

It is with the hope that some light may be thrown on this question of the essential cause of disturbed excitability or stimulus formation that the following case of paroxysmal auricular fibrillation is reported

CASE REPORT

History—I G Hospital No 601, a jeweler, aged 50, born in Russia, was admitted to the hospital Feb 23, 1912, complaining of cardiac palpitation and weakness He knows of no cases of cardiac disease or rheumatism in his family, and the family history is otherwise negative The patient had been a generally healthy man, but always "high strung" and rather nervous He thought that he had neither scarlet fever nor diphtheria in childhood, but since the age of twelve he had frequent attacks of tonsillitis and at the age of 15 typhoid fever When 18 years old he had an apparently rather mild attack of gonorrhea, but there never had been any other venereal affection His wife is living and well, and they have several healthy children He had considerable distress after eating for many years, and had always taken large amounts of food, especially bread, and much tea He used both alcohol and tobacco moderately and had

* Submitted for publication Sept 24, 1913

* From the Hospital of the Rockefeller Institute for Medical Research, New York

never done laborious work He never had rheumatism, but there was pain without swelling or redness in the right shoulder, beginning about eleven years ago and lasting fifteen months He continued his occupation as a jeweler with very little interruption up to a few weeks before admission to the hospital

The present illness began twelve years ago, in 1900, when the patient felt undue fatigue after exertion, and on the following day he had his first attack of cardiac palpitation and weakness, much like that for which he came to the hospital He was in bed for three days at this time He then had no such symptoms for about eighteen months, when the second attack occurred, and since then he has had similar attacks, usually lasting only a few hours, every four to six months Occasionally they have lasted as long as three days The patient thinks that the gastric disturbance, noticed especially after eating, had something to do with causing the attacks of palpitation About eight weeks before admission the patient had the first of the series of attacks which brought him to the hospital The first attack began Dec 22, 1911, in the usual way, with a boring feeling in the epigastrium, a thumping feeling now and then in the heart, and finally a sudden onset of cardiac palpitation There was also a feeling of pressure about the chest, and choking, headache and weakness This attack lasted twenty hours and stopped suddenly. During the next three weeks the patient had two or three attacks of lesser severity, and then, Jan 13, 1912, an attack began which lasted three and a half days This was followed by four days when the patient felt quite well, at the end of which time the symptoms returned and continued for seven days Then he was free from symptoms for ten days, when another attack, lasting a few days, occurred Finally, after the patient had been feeling well for nearly two weeks, the final attack before admission began, and this time in an unusual way, a feeling of palpitation coming and going for a day or so, and then settling down on February 22, the day before admission to what seemed to the patient a severe attack Besides the symptoms that have been mentioned he had moderate pain in the left arm and in the back of the neck He was able to walk into the hospital, but with some difficulty

Physical Examination—Physical examination showed the patient to be a rather poorly nourished, small man, who looked at least ten years younger than his age He was lying quietly and apparently comfortably in bed, but appeared somewhat nervous He was alert and highly intelligent The eyes showed normal pupillary reactions to light and accommodation The fundi were apparently normal except for slight tortuousness to the arteries The tonsils were not enlarged There was slight venous pulsation in the neck The thyroid was not palpable The lungs yielded normal physical signs throughout There were no abnormal pulsations seen over the thorax, while there was a general heave in the region of the left nipple The precordium was not prominent The apex beat was in the fifth space, 10 cm. to the left of the midsternal line The outline of cardiac dulness extended 4 0 cm to the right of the midsternal line in the fourth space, and 11 5 cm to the left of the fifth space The heart sounds were clear throughout, no murmurs being heard The second pulmonic was slightly accentuated, and had a liquid quality The radial pulse was distinctly irregular, and had a rate of about 75 per minute Many of the pulsations were well sustained, but they were irregular in both force and rhythm The actual heart-rate as counted by auscultation over the heart was 126 per minute The arteries were distinctly palpable in the arm, at the wrist and especially at the elbow The temporal arteries were also indefinitely palpable The abdomen was normal Neither liver nor spleen was palpable There was no edema of the extremities Blood, hemoglobin 95 per cent (Sahli) corrected reading = 119 per cent Wassermann reaction was negative The urine showed neither albumin nor casts Its specific gravity was 1 030 The amount was 650 c c in twenty-four hours Electrocardiograms showed that auricular fibrillation was present (Fig 1)

Subsequent Course—Digitalis was begun three days after admission, and in three days distinct slowing of the ventricles had occurred and the arhythmia was much less marked March 1, at 10 30 a m, the patient was bled from an arm vein and 40 c c of blood was obtained At 11 a m he noticed a sudden change in the feeling of the heart beat, and declared that his attack was over The examination revealed that the patient's heart was beating regularly, at first at a rate of 108, and in a few hours at about 90 The outline of relative cardiac dulness extended 4 cm to the right in the fourth space, and 10 cm to the left in the fifth space The heart sounds were clear and of good quality all over the precordium There were no murmurs, but the second aortic sound was accentuated The patient felt immediately distinctly more comfortable, and seemed to be entirely well except for slight weakness Electrocardiograms showed that the normal sequential beat had returned (Fig 2)

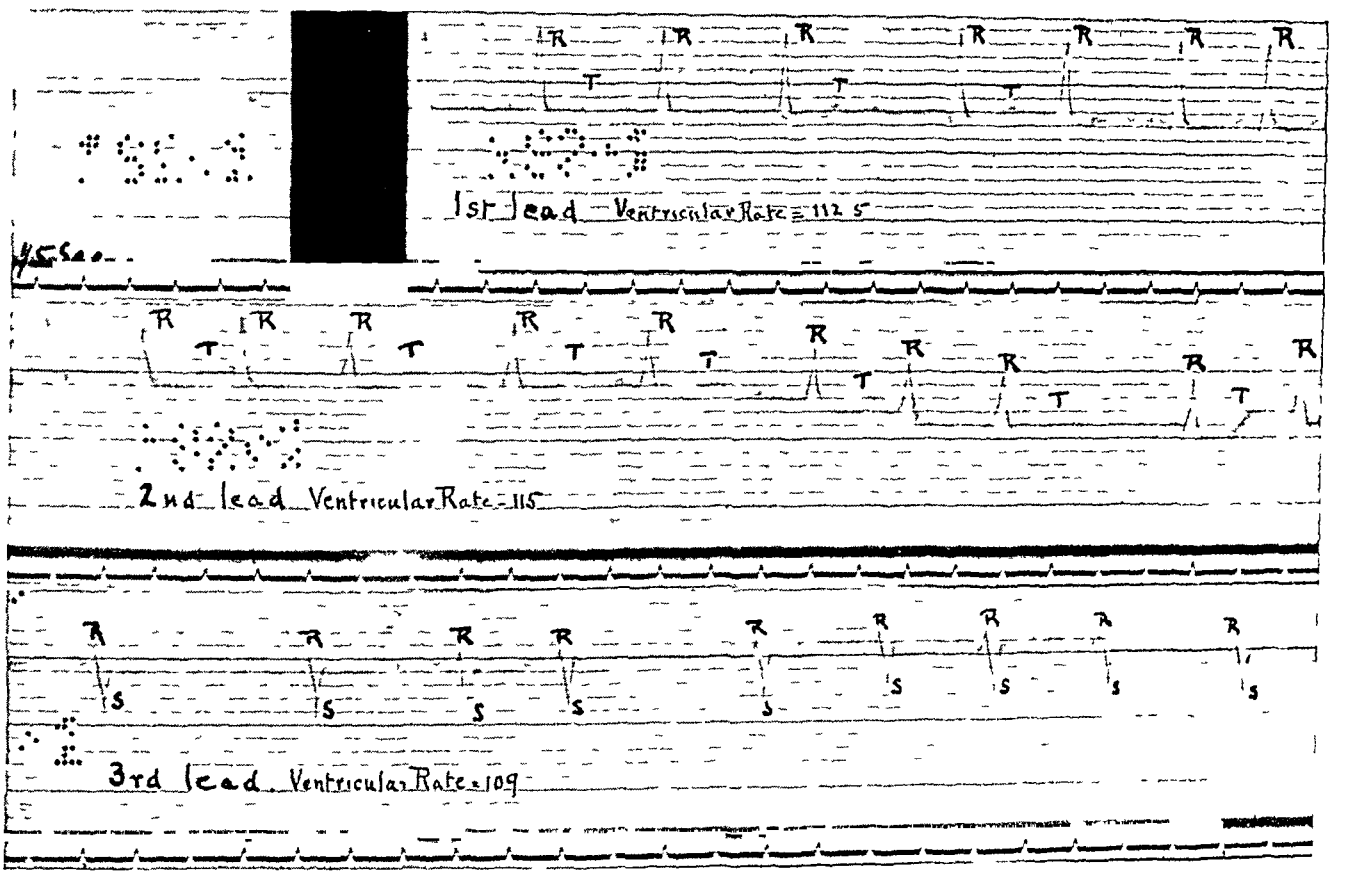


Fig 1—Curves 192 February 25 First, second and third leads, showing auricular fibrillation Ventricular rate averages 112 beats per minute The time marking is always in 1/5 seconds

During the night of March 6 after the heart had been beating normally for about sixty hours, the patient felt an occasional jumping feeling in the epigastrium, which he said usually occurred before attacks, and which led him on this occasion to predict the return of cardiac palpitation During the night it was noticed that cardiac arhythmia existed for a few minutes on several occasions

The heart continued to beat regularly, however, until March 10, when the patient awoke at 1 a m with the sensation of weakness and felt that his heart was beating irregularly When examined at 9 30 a m the typical arhythmia of auricular fibrillation was present, the diagnosis of which was confirmed by

electrocardiograms This arhythmia continued until the afternoon of March 14, nearly five days On this day the patient was restless, worried and especially uncomfortable in the morning, but in the afternoon the heart suddenly, practically instantly, became regular The patient immediately felt much better, and except for muscular soreness and weakness was without symptoms The cardiac examination gave results similar to those obtained during his first period of cardiac regularity His condition remained excellent, and on March 22, after the heart had been regular for eight days, the patient was allowed to sit up out of bed for half an hour March 23, a few minutes before 7 a m, the pulse became suddenly irregular again When seen a little later the patient was comfortable, and the heart, although completely irregular was not beating very rapidly, and nearly all beats produced palpable radial pulsations The heart sounds were clear The arhythmia was still present on March 25, when the patient complained of feeling especially badly with dizziness, weakness and a

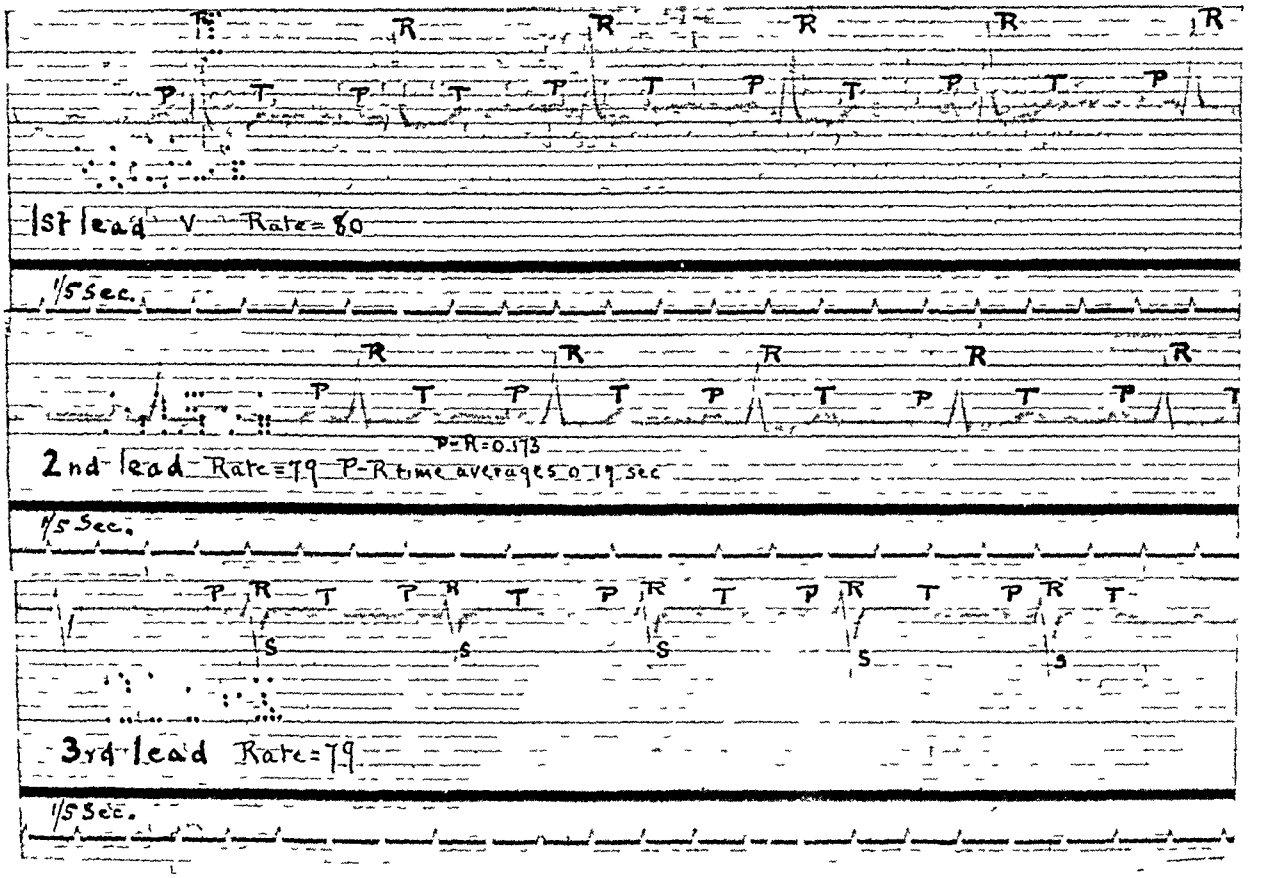


Fig 2—Curves 205 March 4 First, second and third leads obtained during the first period of cardiac regularity The records show that the normal sequential beat is present P-R time, second lead, average 0 17 second Rate averages seventy-nine beats per minute

feeling of helplessness On March 26, at about midnight, the patient awoke to find that his pulse was regular and rapid, and when examined the next morning the heart was beating perfectly regularly as before The outline of relative cardiac dulness extended 2 cm to the right and 11 5 cm to the left in the fourth space, and the heart sounds were as before The cardiac rate was 80 Electrocardiograms again showed that the auricles were beating normally, and were once more properly coordinated with the ventricles The patient seemed in excellent condition without symptoms, and the heart maintained a rate of about 72 for

several days During the night of April 1, however, the patient had pain in the epigastrium and a feeling of suffocation, and on April 2, at 6 a m, his pulse became suddenly irregular When examined at 10 a m, the heart was beating very irregularly, at a rate of about 140 per minute Many beats failed to reach the wrist, and the radial pulse rate was only about 100 per minute The outline of relative cardiac dulness extended 3 5 cm to the left in the third space, and 3 0 cm in the fourth space, and 10 0 cm to the left in the fourth space The electrocardiograms were those of auricular fibrillation From April 2 the cardiac arhythmia continued as long as the patient was under observation The heart rate gradually slowed and became more nearly regular, but electrocardiograms showed that auricular fibrillation was still present when he was discharged on May 23, feeling quite comfortable and well He had been walking about the ward for several days without symptoms The arhythmia continued for several weeks after the patient left the hospital It was subsequently learned that the patient died about two months later with symptoms suggesting acute dilatation

DISCUSSION

During the time the patient was in the hospital he had three periods of nine, nine and six days, when the heart was beating regularly, between which auricular fibrillation occurred for five and four days It was also present during the first six days and during the last fifty-one days in the hospital

The change from one form of cardiac mechanism to another was usually sudden, but premonitory symptoms were sometimes noted by the patient before the onset of the arhythmia These were, as have been described, a feeling of gastric distress and a thumping feeling in the cardiac region which was probably caused by the occurrence of premature ventricular contractions (extrasystoles) The occurrence of such beats was recorded by electrocardiograms several times during periods of fibrillation The actual onset of auricular fibrillation occurred each time after the patient had been asleep, once apparently during sleep On the first occasion the patient awoke at 1 00 a m with a sensation of weakness and felt that his heart was irregular, and the second and third occasions the arhythmia began suddenly at 7 00 a m and 6 00 a m, respectively Muscular exertion and psychic excitement, unless it was in dreams, played, therefore, no rôle in initiating auricular fibrillation Gastric disturbance also did not seem to be a factor at such times Thus, there was no evidence supporting the patient's statement concerning the influence of gastric distress on the cardiac rhythm, which may be a factor in the production of complete arhythmia as v Muller[1] has pointed out

Auricular fibrillation changed to the normal sequential beat three times while the patient was in the hospital The first change occurred at 11 00 a m, half an hour after 40 c c of blood had been withdrawn from an arm-vein, the second during the afternoon following an uncomfortable and restless forenoon, while the third change occurred at midnight,

1 Muller, v The Nervous Affections of the Heart, THE ARCHIVES INT MED, 1908, 1, 1

apparently during sleep The changes were always sudden, and as the conditions under which they occurred were so different, no conclusions as to the possible cause of the changes can be drawn The blood-letting was thought to have been a possible factor, but it was repeated on April 22, after fibrillation had been present three weeks, without any change in the cardiac mechanism The patient stated that the return to the regular rhythm usually occurred when he was quiet

The administration of digitalis may have been a factor in causing the disappearance of auricular fibrillation on two occasions The drug was begun in the form of digipuratum tablets, 0 1 gm , three days after admission, five tablets the first day, decreasing one tablet each day On February 29 the patient was nauseated after having had 1 5 gm of digitalis, and the drug was discontinued at 3 30 p m The fibrillation ceased at 11 00 a m the next morning

The drug was begun again in the same manner on March 11, two days after the onset of fibrillation, and continued for four days, when it was stopped after the sequential beat had returned Small doses of the tincture, 0 2 c c , three times a day, were given during this period of normal rhythm with the idea that it might assist in maintaining the regular beat, but on March 23 fibrillation again set in, and digipuratum was begun as before This was continued for two days, and was discontinued after ten doses, as the patient complained of feeling dizzy and weak Thirty-six hours after drug was discontinued, the normal beat again returned The drug was withheld after the next onset of fibrillation had been present fifteen days, because it was felt that its administration probably had no effect in stopping the auricular fibrillation, and that the sequential beat would return spontaneously As this did not occur, digipuratum was again administered At this time and again later, it was given until nausea occurred, but produced no apparent effect on the auricular fibrillation Digitalis was given primarily not with the idea of influencing the auricular activity, but because the rapid, irregular and insufficient ventricular activity present at the time of admission demanded it In fact, as Mackenzie[2] and others have pointed out, digitalis may be apparently a factor determining the onset of auricular fibrillation In our case fibrillation seemed prone to cease a day or two after the drug had been pushed to its physiological limit and discontinued On one occasion fibrillation came on while small doses of the tincture were being taken

The ventricular activity was favorably influenced by digitalis, and the rapid, irregular heart beat was reduced to a rate of about 80, and became almost regular, while fibrillation continued During the periods of regular cardiac activity the electrocardiograms showed that there was no prolongation of the time of conduction from auricles to ventricles, the

2 Mackenzie Digitalis, Heart, 1910-11, II, 295

P-R time in the second lead records averaging 0 17 seconds This case does not lend support, therefore, to the suggestion put forward by Lewis[3] "that the slowing of the heart, when auricular fibrillation is present and digitalis is given, is due to an increase of a previously existing defect in the conduction to the ventricle of those impulses which are built up rapidly and irregularly in the auricle '

BLOOD-PRESSURE

The relation of changes in blood-pressure to changes in the cardiac mechanism was studied The difficulty of determining the blood-pressure in cases of auricular fibrillation is evident, as the ventricular contractions, varying in force, produce variations in the blood-pressure from beat to beat On account of the fact that exact estimations were impossible, the blood-pressure was not recorded at first, during auricular fibrillation, but later, when the heart was beating more nearly regularly, the pressure was determined by the auscultatory method, at which the strongest beats produced sounds in the brachial artery and also at which practically all beats produced sounds The average of these two readings was considered as the systolic blood-pressure In a similar way two diastolic readings were taken and averaged The blood-pressure readings are seen in the accompanying chart (Fig 3) Although the blood-pressure was constantly high marked changes took place, the systolic pressure varying from 125 to 215 mm Hg The diastolic pressure was more constant, but varied from 85 to 120 mm Hg In the second period of regular rhythm, from March 14 to 23, the blood-pressure rose gradually from 125 mm Hg to 175, when fibrillation again set in The blood-pressure did not fall with the onset of fibrillation, but twenty-four hours later it had fallen 25 mm Hg and remained relatively low until about thirty-six hours after the heart had become regular once more Then the systolic pressure rose to 185 mm. Hg, remained high for three days, and then fell to 130 the day before fibrillation again set in The blood-pressure was taken between 10 00 a m and 11 00 a m each day, and no observations were obtained immediately before and after a change in cardiac rhythm, so the day-to-day readings give no accurate indications as to changes in blood-pressure as a factor in causing changes in the cardiac mechanism On the hypothesis that the high blood-pressure might be a factor in maintaining fibrillation, however, the patient inhaled 0 2 c c of amyl nitrite about three hours after the onset of fibrillation on March 23, at which time the systolic blood-pressure was 180 mm Hg He became nervous and dizzy, but except for the initial rapidity the heart beat did

3 Lewis Auricular Fibrillation and Its Relation to Clinical Irregularities of the Heart Heart, 1909 10, 1, 306

4 Lewis Fibrillation of the Auricles, Its Effects Upon the Circulation, Jour Exper Med, 1912, xvi, 395

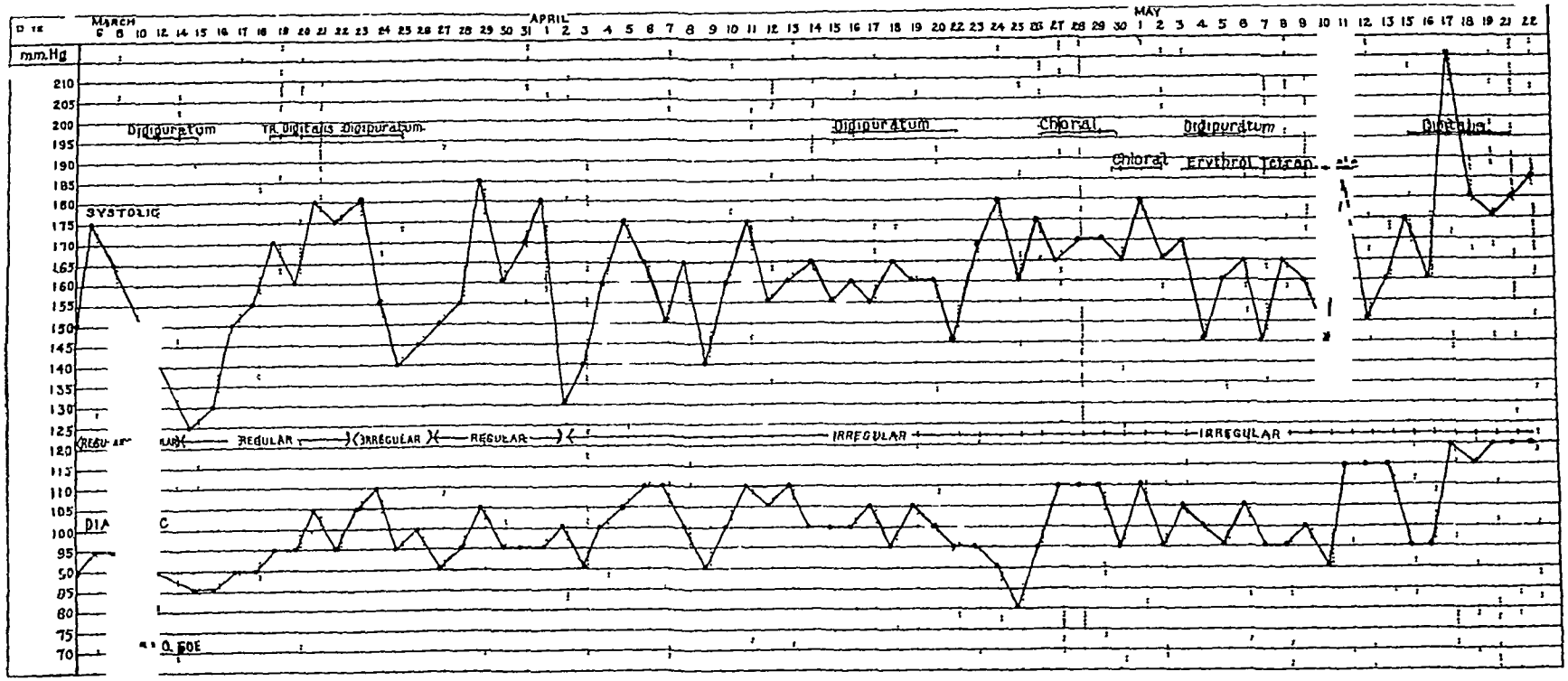


Fig 3—The systolic (upper line) and the diastolic (lower line) blood-pressure The type of cardiac activity and the drug administration are also indicated

not change, and the blood-pressure was apparently not lowered On another occasion (April 22), three weeks after the onset of fibrillation, the systolic pressure fell during the operation of blood-letting to 115 mm Hg without any change in the cardiac mechanism Erythrol tetranitrate and chloral were also administered during fibrillation, but without affecting appreciably the blood-pressure or the cardiac mechanism On the other hand, the systolic pressure reached 215 mm Hg on one occasion during fibrillation without any change in the beat It may be said, therefore, that no relationship between changes in blood-pressure and changes in the cardiac mechanism can be made out The blood-pressure varied greatly, both during auricular fibrillation and during the normal rhythm, but the blood-pressure was maintained generally at a high level, regardless of the form of cardiac activity present These blood-pressure observations are in accord with those made by Lewis[4] on the experimental animal, in which he found that the onset of fibrillation was usually accompanied by a fall of blood-pressure, but the effect was inconstant The circulation tended to accommodate itself to altered conditions, and to return to the same blood-pressure as that observed before the onset of fibrillation

The effect of pressure on the neck over each vagus nerve was recorded by electrocardiograms several times, both during the normal rhythm and during fibrillation (Figs 4-7) The abnormal susceptibility of the heart to mechanical stimulation of the vagi was striking The length of time pressure was made over each nerve and the length of the responding pause may be tabulated as follows

AURICULAR FIBRILLATION (FIGS 3 AND 4)

	Pressure Over Right Vagus Seconds	Ventricular Stoppage Seconds	Pressure Over Left Vagus Seconds	Ventricular Stoppage Seconds
	3 0	3 3	3 4	4 8
	3 4	4 0	3 1	4 0
	2 8	4 0	3 6	4 4
	3 0	3 1		
	3 0	3 4		
Average	3 04	3 56	3 37	4 42
Proportion	1	1 17	1	1 22

NORMAL BEAT (FIGS 5 AND 6)

	Pressure Over Right Vagus Seconds	Ventricular Stoppage Seconds	Pressure Over Left Vagus Seconds	Ventricular Stoppage Seconds
	1 4	3 2	2 0	3 6
	1 9	3 0	2 0	4 0
	1 9	3 0	2 0	1 9
Average	1 73	3 06	2 0	3 17
Proportion	1	1 77	1	1 53

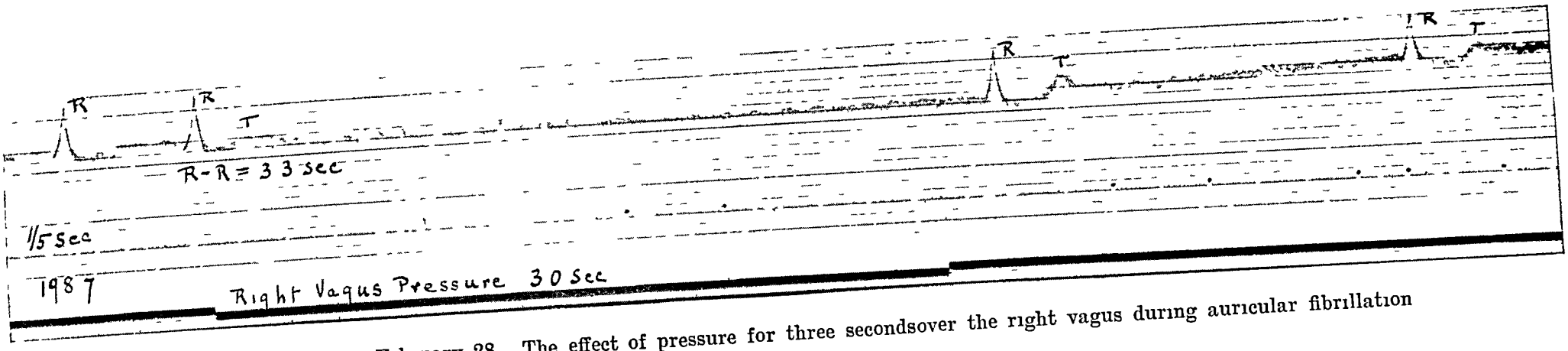


Fig 4—Curve 198 7 February 28 The effect of pressure for three secondsover the right vagus during auricular fibrillation

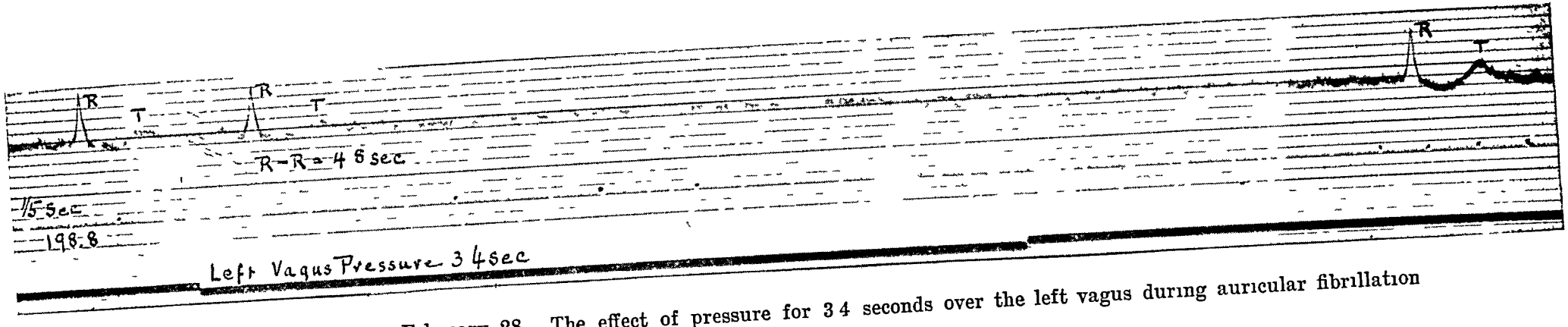


Fig 5—Curve 198 8 February 28 The effect of pressure for 3 4 seconds over the left vagus during auricular fibrillation

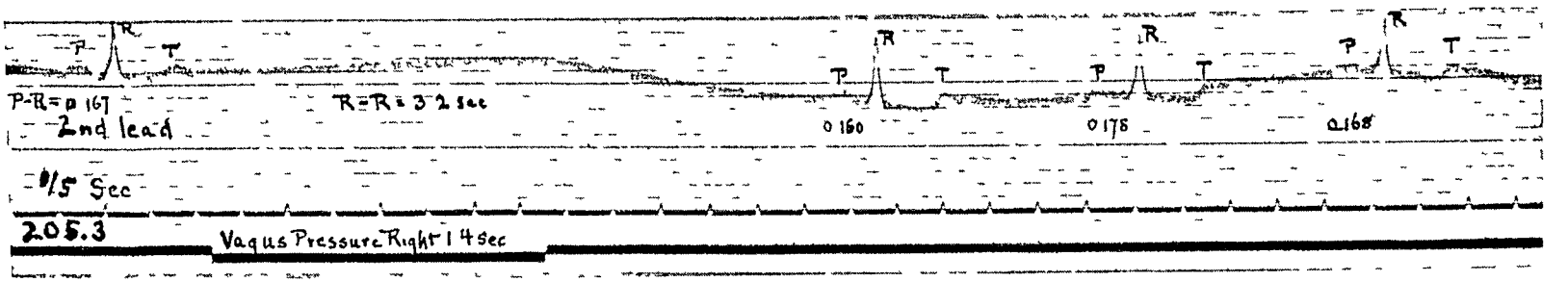


Fig 6—Curve 205 3 March 4 The effect of pressure for 1 4 seconds over the right vagus on the normally beating heart

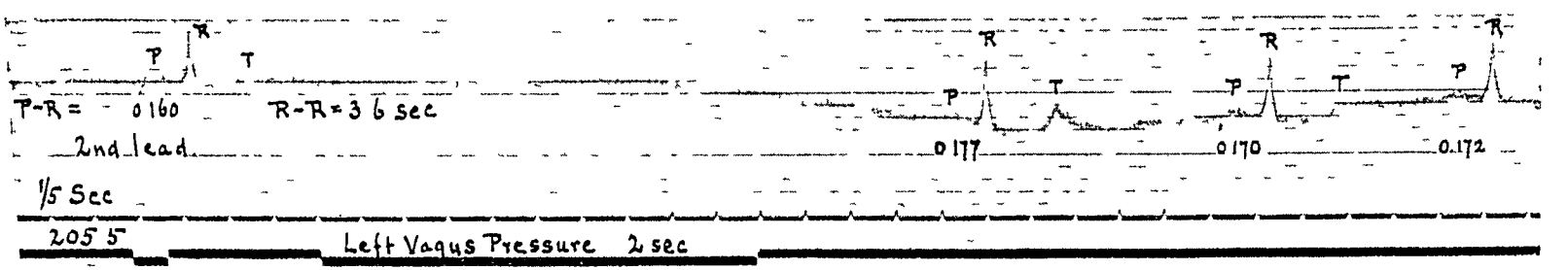


Fig 7—Curve 205 5 March 4 The effect of pressure for 2 seconds over the left vagus on the normally beating heart

Thus it is seen that neither during auricular fibrillation nor during the normal beat can any definite difference be made out between the effects of right and left vagus pressure, such as can sometimes be demonstrated in man (Robinson and Draper[5]), and as can be almost constantly shown with faradic stimulation of the nerves in the dog (Cohn,[6] Robinson[7]) The ventricles were stopped for a longer time proportional to the length of pressure when the heart was beating normally than when auricular fibrillation was present, whether the left or the right nerve was pressed on

The mechanism responsible for the ventricular pauses which followed vagus pressure during auricular fibrillation is apparently different from that which brings about the stoppage of the normally beating heart The electrocardiograms indicate that the activity of the fibrillating auricles was unaffected by pressure over the vagi, while they show that the normally beating auricles were brought to rest by this procedure The manner by which vagus pressure brings about the long cessation in the ventricular activity in cases of auricular fibrillation has been discussed by Robinson and Draper,[5] and it was concluded that the ventricular pauses are apparently due to the blocking of stimuli from the auricles There was no evidence as to whether this striking effect depends on an increased susceptibility of the conducting fibers to vagus stimulation or whether it depends on the stimuli from the fibrillating auricles being of such a nature that only slight lowering of the conductivity succeeds in blocking them The possibility must also be considered of some quantitative change in the auricular activity which is not to be detected in the electrocardiograms, but which makes it incapable of stimulating ventricular contractions

When the heart was beating normally there is practically no evidence that pressure over either vagus nerve lowered the conductivity, as the P-R time was never prolonged beyond 0 21 second after the cardiac stoppage in any of the records The marked effect on the rate of the whole heart is in sharp contrast to the effect on conduction It may be, however, that this effect on rate is responsible for the lack of effect on conduction, for during the cardiac standstill the conductivity of the heart is freed from the influence of functional fatigue, and so has its normal capabilities at the end of the cardiac pause The harmonizing of the effects of vagus pressure on the heart during auricular fibrillation with that observed when the heart was beating normally is difficult

5 Robinson and Draper I Studies with the Electrocardiograph on the Action of the Vagus Nerve on the Human Heart, Jour Exper Med, 1911, xiv, 217, II Studies with the Electrocardiograph on the Action of the Vagus Nerve on the Human Heart, Jour Exper Med, 1912, xv, 14

6 Cohn Jour Exper Med, 1912, xvi, 732

7 Robinson The Influence of the Vagus Nerves on the Faradized Auricles in the Dog's Heart, Jour Exper Med, 1913, xvii, 429

The marked susceptibility of the heart to mechanical stimulation of the vagi may have been another expression of the cardiac abnormality which caused the auricular fibrillation The hearts of patients with auricular fibrillation are often markedly affected by pressure over the vagi and an abnormal response to this procedure by a normally beating heart may possibly indicate that auricular fibrillation is more likely to occur in it than in a heart less affected by vagus pressure It is difficult to say whether this abnormal response indicates a so-called heightened "vagus tone" or whether the heart itself is responsible for the marked response A series of experiments were undertaken with the hope primarily that some new light might be thrown on the question of the relation of vagus activity and auricular fibrillation, but the results of the experiments[7] do not afford any positive evidence as to the rôle the vagi may play as a causative factor of auricular fibrillation in man The dog's auricles may be thrown into fibrillation and tachycardia by direct faradization as readily after the vagi are cut as before, but vagus stimulation holds the auricles in their abnormal state of activity after it has been once set up by faradization

The effect of atropin in our case was observed only during fibrillation, and after 0 9 mg atropin had been given hypodermically the ventricular rate increased markedly from 84 to 158 beats per minute, recorded by an electrocardiogram taken forty minutes after the administration of atropin The waves of fibrillation were not so distinct in the records obtained during the rapid ventricular rate as before The great increase in ventricular rate following atropin indicates that the vagi were constantly strongly active at this time in controlling the ventricular rate A desire not to disturb the normally beating heart prevented the use of atropin during the periods of regular rhythm

A series of electrocardiograms were made directly from various points on the chest wall during the normal beat and during fibrillation The technic described by Lewis[8] was followed, in the hope of gathering further evidence regarding the significance of the waves of auricular fibrillation in the electrocardiograms The results were unsatisfactory, as neither the P waves nor the waves of auricular fibrillation were distinct

When this patient is considered from an anatomical rather than from a physiological viewpoint it is found that the heart is normal as regards its size, position and the character of the heart sounds No valvular murmurs were ever heard, and although the second aortic sound was somewhat accentuated, the muscle sounds were of good quality The outline of cardiac dulness was measured repeatedly, and no changes in

8 Lewis The Origin of the Electric Oscillations and the Direction of Contraction of the Ventricle in Instances of Complete Irregularity of the Heart (Auricular Fibrillation), Quart Jour Med, 1911, v, 11

size as those observed by Lewis and Schleiter[9] could be made out, as an accompaniment of the changes in mechanism A slight increase in the size of the heart between the time of admission and of discharge was noted, however, the total width increasing 1 cm during this time

The constantly high blood-pressure and the palpable arteries in the arms and temporal regions led to the diagnosis of arterial sclerosis The urine gave no evidence of diseased kidneys, as the usual tests were repeatedly negative except for several hyaline casts on two occasions The functional activity of the kidneys was tested May 7 by the technic of Rowntree and Geraghty,[10] 6 mg of phenolsulphonephthalein being injected intramuscularly The first appearance of the drug in the urine was not definitely determined, but was estimated at about ten minutes During the first hour 36 8 per cent and during the second hour 21 9 per cent, or a total for two hours of 58 7 per cent of the drug was excreted Thus the functional activity of the kidneys was but slightly below the normal limits of excretion (40-60 per cent the first hour, and 60-85 per cent in two hours) The sole definite anatomical lesion, therefore, is apparently arterial sclerosis

Arterial sclerosis is frequently a prominent characteristic of cases of auricular fibrillation Thus Lea[11] found it present in 57 1 per cent of the non-rheumatic cases in his series No definite change in the cardiac muscle has been described in these cases with sufficient frequency to allow any conclusions to be drawn as to an anatomical basis for auricular fibrillation and the post mortem examinations of some of the hearts in which fibrillation had been present reveal hearts which are anatomically sound (Gossage and Hicks[12]) Lewis[3] believes that it is changes in the walls of the auricles themselves which probably cause fibrillation, and he has expressed the opinion that anemia of the auricular walls may be a factor in setting up fibrillation Changes in the blood-supply of the auricular walls may well have been an important factor in causing the attacks of fibrillation in our patient The evident arterial sclerosis and the high but markedly changing blood-pressure might readily have caused abnormalities in the cardiac blood-supply, which not only rendered the auricles more prone to fibrillation and abnormally susceptible to vagus stimulation, but also furnished the immediate cause of an attack of auricular fibrillation by a temporary diminution of the cardiac circu-

9 Lewis and Schleiter The Relation of Regular Tachycardias of Auricular Origin to Auricular Fibrillation, Heart, 1912, iii, 173

10 Rowntree and Geraghty An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, Jour Pharm and Exper Ther , 1909-10 i, 579

11 Lea Some Points in Relation to the Etiology of Auricular Fibrillation, Quart Jour Med , 1911, iv, 423

12 Gossage and Hicks On Auricular Fibrillation, Quart Jour Med , 1913, vi, 435

lation There is very little in support of this hypothesis, but arterial sclerosis and high blood-pressure have been prominent in several cases of transitory auricular fibrillation which have been observed Thus Hornung[13] reports three cases in which these abnormalities were prominent features, and arterial sclerosis was also mentioned in two of Fox's[14] six cases The case reported by Lewis and Schleiter, although only 28 years old, had somewhat thickened arteries, but on the one occasion recorded the blood-pressure was low

The paroxysms of auricular fibrillation may occur over a period of many years, and our patient had them apparently for twelve years One of Hornung's cases suffered with attacks of cardiac arhythmia for five years and another for at least ten, while one of the cases reported by Fox, the case originally observed by Cushing and Edmunds, had attacks for over twenty years These cases, therefore, do not always deserve the bad prognosis which is usually accorded to auricular fibrillation They may have anatomically normal hearts and be closely allied to cases of paroxysmal tachycardia, as Lewis and Schleiter point out The prognosis may not necessarily be more grave than in tachycardia However, the auricular fibrillation tends to become permanent, as Fox's cases well illustrate, and this is what happened in our case, as fibrillation was present during the last two months that he was in the hospital, and continued until his death about two months after his discharge There is, therefore, apparently a group of cases which may be termed paroxysmal auricular fibrillation, in which arterial sclerosis may be an underlying cause of the abnormal cardiac activity and in which attacks of fibrillation may recur over a period of many years in patients with anatomically normal hearts, without great impairment to the general health

SUMMARY

A case is reported in which transient attacks of auricular fibrillation were observed and recorded by electrocardiograms These attacks had apparently occurred over a period of twelve years, and auricular fibrillation became permanent while under observation No definite cause could be discovered as initiating the attacks No definite relation could be established between changes in blood-pressure, which was always abnormally high and variable, and the changes in the cardiac mechanism The heart was strikingly susceptible to pressure over each vagus nerve, both during fibrillation and during the normal cardiac activity This abnormality may be another expression of the changes causing auricular fibrillation Electrocardiographic records of these vagus effects were obtained

13 Hornung Uber atypische Tachykardische Paroxysmen, Deutsch Arch f klin Med, 1907 xci, 469

14 Fox The Clinical Significance of Transitory Delirium Cordis, Am Jour Med Sc, 1910, cxl, 815

The heart of the patient gave no signs of an anatomical lesion, but arterial sclerosis, a frequent accompaniment of auricular fibrillation, was present The presence of this lesion and the high, inconstant blood-pressure suggest the possibility that changes in the cardiac blood-supply may have been the underlying causative factor in this case Other cases showing attacks of auricular fibrillation occurring over a period of years have been reported in which high blood-pressure and arterial sclerosis were prominent features These cases seem closely allied to paroxysmal tachycardia, and although the auricular fibrillation tends to become permanent, the prognosis for them is not necessarily so grave as that which is given in ordinary cases of auricular fibrillation with outspoken cardiac lesions

1806 Locust Street

THE MUTUAL ACTION OF CERTAIN DIGESTIVE FERMENTS *

J H LONG, ScD, AND G W MUHLEMAN, MS
CHICAGO

In the earliest days of the investigation of some of the important soluble ferments, or enzymes, the question of their behavior toward each other came up for study In the human body we have to consider the action of the salivary, the gastric, the pancreatic and the intestinal ferments, and the behavior of each one of these may be modified not only by the reaction of the medium in which it acts, but also by the presence of others Since the activity of the salivary diastase is not confined to the mouth, but is continued to the stomach, the effect of the hydrochloric acid and pepsin present has naturally been the subject of frequent studies Practically, the behavior of the acid alone has real importance, since the ptyalin is so sensitive to the acid that even minute traces are sufficient to render it inert That pepsin, also, may have an effect on ptyalin, or ptyalin on pepsin, are academic questions of little practical importance, and of no importance at all in the present inquiry

Much more importance attaches to the inquiry, What is the action of the gastric juice, that is, of pepsin plus hydrochloric acid, on the group of ferments furnished by the pancreatic secretion? In the normal conditions obtaining in the animal body, and especially in the human body, it is evident that there is no interference of the one with the other Passing down into the intestine remains of the gastric secretion, with acid reaction, become speedily neutralized and brought into a condition in which pepsin, if any of it remains, cannot act on either proteins or ferments The question, however, becomes a very different one when we seek to determine the action of the pepsin, or the whole gastric juice, on artificial pancreas products given by the mouth, and which therefore must pass through the stomach

This is the question which comes up in studying the behavior of the commercial product known as pancreatin, and we are permitted to ask about the action of these pancreas ferments, on the one hand, on pepsin, and, on the other hand, of the action of pepsin on them The question is an old one and has been in the literature many years We find that in his classic researches on the preparation and properties of trypsin Kuhne, in the seventies of the last century, touched on it, and many subsequent investigators have dealt with some phase of it

* Submitted for publication Jan 5, 1914

In view of the wide-spread use of pancreatin the inquiry has no little practical importance, and it comes to us now through questions raised in the investigations of the Council on Pharmacy and Chemistry on pancreas preparations which are supposed to resist the action of the gastric juice and exhibit their characteristic effects after passing through the stomach

That "pancreatin" is much weakened in its action by contact with pepsin is often affirmed and perhaps generally believed, if one can judge by the somewhat vague statement of the Pharmacopeia, Eighth Revision "The digestive power of pancreatin is injured by contact with pepsin in solution" Not only is this statement vague, but also it may not be altogether in accord with facts The vagueness, however, is in a measure associated with the vagueness in the definition of pancreatin itself Under the name "pancreatin" we understand a product obtained in some manner from the pancreas of the hog or ox, but as to the method of preparation or the ferment content of the article there is not much agreement. It is usually assumed that all the pancreas enzymes are present, but in the method of testing official in our Pharmacopeia only one of them is practically recognized and the content of this is assumed to be small As to whether the determined value found for the amylopsin is to be taken as a measure of the content of the other ferments, or not, nothing is said

What is called pancreatin is often not much more than fat-free ground pancreas Perhaps this is usually the case, but in the older view, and that still held by many writers, pancreatin should be obtained by some process of *extraction* from the pancreas, and precipitation or concentration of the extract The question of expense seems to prevent the manufacture in this way, as a rule, and there would be little or no advantage in the matter of stability

Pancreas powders, or extracts, are in general very low in the lipase enzyme, and in the laboratory as well as in the manufacturing scale it is difficult to secure a permanent product which has much fat-splitting power In dealing with "pancreatins," or other commercial products of this class, we are practically concerned with their amylolytic or proteolytic power only To what extent, if at all, are these ferment activities injured by contact with gastric juice or its pepsin-acid equivalent? And in turn, have these ferments any action on pepsin?

These are the questions we have attempted to answer through a series of experiments

1 THE ACTION OF HYDROCHLORIC ACID AND PEPSIN ON AMYLOPSIN

This is the statement of a problem which is more ideal than practically workable, since neither the amylopsin nor the pepsin is obtainable

in a pure or nearly pure condition Both of the ferments, as we obtain them commercially, or as we prepare them in the laboratory, are associated with relatively large amounts of matter which, while inert as far as ferment action is concerned, may be very important in modifying ferment action This is true of the phosphate and other salt content of the ferment products

For the purposes of this inquiry the question is asked what is the behavior of the best obtainable laboratory or commercial products toward each other? and that is the problem to be discussed

It requires but a brief study to show that the starch-converting ferment from the pancreas, as well as that from malt, is very sensitive toward acids

Before touching the question of the pepsin behavior, it is therefore necessary to fix the limits of the acid action, as this, rather than pepsin, may be the main factor in the case The Pharmacopeial statement is misleading in this respect Various statements are found in the literature concerning the acid concentration which stops the amylolytic action of the ferments,[1] and these statements are often in conflict because the experiments on which they were based were doubtless made with products of very different degrees of purity, especially in respect to salt content The phosphates of sodium, potassium, calcium and magnesium which are associated to some extent with most of these ferment products are important factors in modifying the action of added hydrochloric acid, and it must not be forgotten that the binding power of protein for acid is another factor

To eliminate these factors as far as possible we have sought to find the behavior of the acid by using glycerol extracts of the ferment, as well as a considerable number of dry products

These points, then, are to be considered under the general topic of pepsin-hydrochloric acid-amylopsin action

A Action of dilute acid on glycerol extracts and dry products from the pancreas

B Possible action of pepsin, alone, on these products

C Action of pepsin and acid on the same products

The discussion of these problems must be preceded by an explanation of the methods of investigation employed, which, for the present purpose, need be but brief

1 See, for example, the quotations in the work of Green, The Soluble Ferments and Fermentation, and the work of Oppenheim, Die Fermente und ihre Wirkungen, also Effront, Die Diastasen

A ACTION OF ACID ON GLYCEROL EXTRACTS

General Conditions

Glycerol extracts of the pancreas have been used by many observers, and in our own laboratory for years The activity of such extracts and the best conditions for preparation have been most clearly pointed out by Vernon in a series of papers which have gone pretty fully into this phase of the subject [2]

In our work the pancreas extracts were made by mincing the organs very fine and macerating through about three weeks with twice their weight of pure glycerol The mass, thick at first, grew much thinner in time from the evident partial digestion Finally the liquid was separated pretty thoroughly from the suspended particles by straining and by centrifugalization in a powerful machine capable of taking eight tubes of 100 c c each at one operation In this way we secured about 1,600 c c of a cloudy liquid which deposited but little on standing This was extremely active in amylopsin and trypsin, although prepared with careful exclusion from contact with any portions of the intestinal mucosa Even without the addition of salt the liquid was markedly active on starch, but the addition of the salt brought an increase in the activity. In all our experiments aqueous dilutions of this extract were used, in some cases a 1 10 dilution and in other experiments a 1 50 dilution, filtered

Vernon and others who have worked with such extracts state that they retain their activity through months With water solutions, however, the case is quite different, and in a 1 50 dilution, or even in a 1 10 dilution there may be a very marked loss of activity in from ten to fifteen hours, as when the solutions are allowed to stand over night The loss of power is much increased by a slight elevation of temperature and at 40 C may be very decided

If, however, the dilutions are made with salt water in place of pure water, the stability is found to be much greater These facts were illustrated by a large number of experiments made with some commercial pancreatin preparations, as well as with glycerol extracts, in an effort to find an explanation for the great variability in the relative strengths of the market preparations It was found necessary, therefore, to use the water solutions of glycerol extracts as soon as possible after preparation in order to secure the maximum activity This precaution is recommended by several manufacturing firms in methods given for testing their pancreatins

2 Vernon, H M These papers deal with tryptic as well as diastatic action See Journal of Physiology, 1901, xxvi, 405, 1901, xxvii, 269, 316, and in other places

The value of diastase preparations is practically determined by their action on starch paste To secure definite and constant results much depends on the method employed in making the paste, and for details on this point we refer to the general scheme as followed in this laboratory and described by Johnson and one of us[3] in a recent publication

Method of Observation

In following out the action of diastases on clear starch paste made up usually to the strength of 10 gm of anhydrous starch to the liter, we employ the form of the iodin test worked out in this laboratory and reported by Johnson[4] In the digestions, always at 40 C, we carry the reaction to the colorless end-point, when 5 drops of the digesting fluid are added to 5 c c of a dilute iodin solution, made by dissolving 1 gm of iodin and 2 gm of potassium iodid in 125 c c of water and diluting 2 c c of this solution to 1 liter, finally, for use

When the dilute starch paste is added to this iodin solution the blue color comes out sharply As digestion proceeds the blue changes to a purple tint, then a purplish red, a rather deep red, a light red or reddish, a yellowish shade and finally colorless, or at most the faint shade of the weak iodin solution It may be advantageous to use even a weaker iodin to note the final tint, but usually we do not do this When used with pancreatic preparations Sherman[5] objects to this method of following the reaction because of the appearance of the red shades But it is just this appearance and the gradual changes which constitute the most desirable features of the iodin test Its advantages have been pointed out by others[6] The conversion of starch to sugar is far from being an immediate process, and whether the dextrins are definite and sharply definable bodies or not, we must admit that there are various stages in the change from the original starch to the final malt sugar The purple color which succeeds the blue corresponds to the so-called amylodextrin of some authors, and its appearance is a valuable indication of the progress of the action This stage is followed by that of other mixtures, not definite, of course, but all clearly suggesting the rapidity of the transformation It is desirable to employ a method in which this transformation may be conveniently followed, and this is true of the iodin method as used by us At the end we have just as much information as may be derived from the copper reduction tests, which we have often used for controls, and much additional information, besides, which may be lost by making the copper reduction tests in the presence of an excess of starch In tech-

3 Long and Johnson Jour Am Chem Soc, 1913, xxxv, 896

4 Johnson Jour Am Chem Soc, 1908, xxx, 798

5 Sherman and Colleagues Jour Am Chem Soc, 1910, xxxii, 1081

6 See Roberts Proc Royal Soc, 1881, xxxii, 145, Detmer Ztschr f physiol Chem, 1882, vii, 1

nical inquiries as to the value of relatively strong diastase preparations the copper methods are undoubtedly the most satisfactory, but in a strictly physiological study, where the time element is important, certain advantages seem to rest with the iodin process

With this general discussion of the preparation of the materials and the method of testing we are ready for a description of the experiments carried out to test the behavior of weak acids toward the starch-amylopsin reaction.

Like the other actions, that of acids on the starch-digesting ferment of the pancreas has been much discussed It seems desirable to bring out certain conditions here which have not been clearly recognized, although they have been in part pointed out by previous writers

Amylopsin is much more sensitive to the action of traces of acids than to alkalies, and the final effect is more or less dependent on the presence of salts, which may not only serve to stimulate the catalytic action of the ferment, but also act to depress the activity of the acid hydrogen in its paralyzing behavior Attempts have been made to show that diastase absorbs gaseous hydrochloric acid through some other than a basic property, and that a large part of the gas may be removed by suction without having fully destroyed the ferment Panzer[7] has published a lengthy investigation on this subject, but the weight of acid added to the ferment preparations was in all cases so large as to permit no conclusion concerning the behavior of the small acidities which really come into consideration where the physiological action is concerned The same is true of the experiments where aqueous acid was used with gram or half-gram weights of the ferment In one case 1 gm of a certain diastase preparation and 0 06775 gm of hydrochloric acid, made up to about 80 c c, stood an hour and was then neutralized with sodium hydroxid and diluted to 100 c c Portions of this dilution were then allowed to act on starch paste, the iodin and sugar tests being made from time to time The figures given show a greatly diminished diastatic conversion, as one might expect from the rather high acid concentration In discussing this and other experiments, the author states that the small amount of salt formed in the neutralization cannot be in any way responsible for the modification of the diastatic action, since such small quantities are of no significance This conclusion does not appear to be warranted by facts already referred to, which will be brought out below. The salt here is undoubtedly of very considerable importance

In our tests we considered the behavior of those small weights of acid which have practical significance Small definite volumes of the glycerol extracts, diluted, were mixed with known weights of acid, and

7 Panzer Ztschr f physiol Chem, 1912, lxxxii, 276

the mixtures made to constant volumes These were then incubated at 40 C through a period of half an hour, usually, after which a constant volume of the 1 per cent starch paste was added and the incubation continued From time to time drops were taken from the incubating mixtures to test for the disappearance of the iodin-starch reaction which would show the conversion of the starch to sugar or forms of dextrin which do not react with iodin The rapidity of this conversion is a measure of the activity of the amylopsin present As the action of the acid becomes evident, the time required to convert all the starch to products not giving a color with iodin is lengthened

Two conditions in the last steps of the reaction may be noticed After the incubation of the ferment and acid mixture the starch may be added directly and a new incubation made, or, after the preliminary incubation, a little weak alkali may be added to neutralize the acid before mixing with the starch paste In the first case the starch digestion takes place in a slightly acid medium, while in the second it is neutral Both lines of observation were followed

In all cases some salt was added to the amylopsin extracts, to bring them all up to the degree of maximum activity which may be secured in this The importance of this in comparative tests has been frequently pointed out

EXPERIMENT 1.—Ten c c of a glycerol extract of pancreas made by diluting the original thick extract with water in the proportion of 1 49 One hundred mg of sodium chlorid added Amounts of hydrochloric acid as shown below were added from dilute solution and the whole diluted to 50 c c for each test, carried out in 200 c c flasks Incubated thirty minutes at 40 C and added to each mixture 50 c c of 1 per cent starch paste Incubated again and withdrew, from time to time, 5 drops from each flask for iodin tests

In a control flask no acid was used, and tests made with this at the end of three minutes showed complete absence of color, indicating total conversion of the starch In five other flasks, with 1, 3, 5, 7 and 9 mg of acid there was no perceptible conversion, as a strong iodin reaction was found in each case, even after an hour of incubation with the starch In a parallel experiment the acid, after the preliminary incubation of thirty minutes, was neutralized with sodium bicarbonate In the following incubation with starch paste the same results were obtained as before

EXPERIMENT 2.—Ten c c of a 1 10 dilution of the glycerol extract were taken for each test and with 100 mg of salt diluted to 50 c c Each test flask contains, then, in the 50 c c 1 c c of the original glycerol extract, and weights of acid as in Experiment 1 After thirty minutes incubation at 40 C 50 c c of 1 per cent starch paste was added to each flask and final incubation began In the control flask, with no acid added, the starch reaction disappeared in three minutes In a flask with 1 mg of hydrochloric acid the starch reaction began to fade in less than six minutes, was weak in ten minutes, and absent in an hour With stronger acid concentrations, 3 mg and above, the starch reaction was always sharp, even after one hour

EXPERIMENT 3.—Conditions same as in 2, but twice as much of the glycerol extract present In the flask with 1 mg of acid present the starch reaction disappeared in the same manner as before, but with the concentrations of 3, 5, 7 and 9 mg of hydrochloric acid there was a perceptible change in the starch reaction in an hour The blue color had given place to purple

In this last experiment there is a suggestion that with larger initial amounts of the pancreas ferment present there is greater practical resistance to the acid Some of the ferment seems to have escaped destruction and is able to bring about a slow conversion of starch In the following experiment still more of the ferment was used

EXPERIMENT 4 —For each test 5 c c portions of the original glycerol extract were diluted with water to 50 c c, receiving at the same time weights of acid as given in Table 1 and 100 mg of salt in each case After the preliminary incubation of thirty minutes at 40 C, 50 c c of paste was added to each flask and the final incubation started The results are shown in Table 1 Complete destruction of the starch, as shown by absence of iodin reaction, is indicated by +, when there is no conversion of starch this in indicated by — Partial conversion is indicated by ∓, with this sign to indicate a progressive change →+

TABLE 1 —DIGESTION OF STARCH WITH PANCREAS EXTRACT PREVIOUSLY INCUBATED WITH HYDROCHLORIC ACID AND SALT (EXPERIMENT 4)

	Time of Incubation, Minutes					
HCl Mg	3	6	10	15	30	45
0	+	+	+	+	+	+
1	+	+	+	+	+	+
3	+	+	+	+	+	+
5	+	+	+	+	+	+
7	∓	+	+	+	+	+
9	∓	→+	+	+	+	+
11	∓	→+	→+	→+	→+	→+

With this stronger concentration of enzyme, its destruction appears to be very much delayed The results seem to suggest that the effect depends not only on the acid concentration, but on the enzyme concentration also In other words, a given weight of acid will destroy or inhibit the action of a given amount of enzyme only A weight combination between the two is suggested in this way, but there is another possibility which must be noticed, and that is that the increasing weight of inert protein substance added with the largest weights of ferment binds to some extent the acid and weakens its action

The amount of protein present, however, is in no case sufficient to hold enough of the acid to effect a marked change in its concentration Certain salts present may have a larger influence on the behavior, as will be pointed out below

A moment's consideration will suggest that the absence of digestive power on the part of the ferment, as shown by the starch test made, may be explained in two ways first, the ferment may be destroyed in the incubation process, or second, the ferment may not be destroyed, but its activity toward the starch may be hindered by the presence of the acid It is well known that larger quantities of acid will stop conversion of the starch, and possibly with the smaller quantities here employed this may take place, without involving the actual destruction of the enzyme Some

experiments with an active commercial pancreatic diastase throw light on this point This product contains considerable chlorid, from the process of manufacture

EXPERIMENT 5.—Pancreas powder, 500 mg to 100 c c plus 3 mg of hydrochloric acid Incubated 50 c c, *a*, through thirty minutes at 40 C, and allowed the other 50 c c *b*, to stand thirty minutes at room temperature Then mixed small volumes with 50 c c of starch paste, 1 per cent, incubated again, and tested after the intervals as shown in Table 2 The degree of conversion is indicated as in 4

EXEPRIMENT 6.—Conditions same as in 5, but 6 mg of hydrochloric acid was present in the 100 c c with the 500 mg of pancreas powder (Table 3)

EXPERIMENT 7.—Conditions as in 5 and 6, but 12 mg of hydrochloric acid present in the 100 c c for the preliminary incubation (Table 4)

TABLE 2.—DIGESTION OF STARCH WITH PANCREAS EXTRACT PREVIOUSLY INCUBATED WITH 3 MG HYDROCHLORIC ACID (EXPERIMENT 5)

	Time of Action on Starch, Minutes						
Ferment Solution	*a*					*b*	
c c	5	10	20	40	60	5	10
4	—	→+	→+	+	+	+	+
6	—	→+	→+	+	+	+	+
8	—	+	+	+	+	+	+
10	—	+	+	+	+	+	+

TABLE 3.—DIGESTION OF STARCH WITH PANCREAS EXTRACT PREVIOUSLY INCUBATED WITH 6 MG HYDROCHLORIC ACID (EXPERIMENT 6)

	Time of Action on Starch, Minutes							
Ferment Solution	*a*					*b*		
c c	5	10	15	25	35	5	10	15
4	—	—	—	—	—	→+	+	+
6	—	—	—	—	—	+	+	+
8	—	—	—	—	—	+	+	+
10	—	—	—	—	—	+	+	+

TABLE 4.—DIGESTION OF STARCH WITH PANCREAS EXTRACT PREVIOUSLY INCUBATED WITH 12 MG HYDROCHLORIC ACID (EXPERIMENT 7)

	Time of Action on Starch, Minutes							
Ferment Solution	*a*					*b*		
c c	5	10	15	25	35	5	10	15
4	—	—	—	—	—	→+	+	+
6	—	—	—	—	—	+	+	+
8	—	—	—	—	—	+	+	+
10	—	—	—	—	—	+	+	+

With the smallest acid concentration, 3 mg of hydrochloric acid to 100 c c, it is seen that the preliminary incubation does not completely destroy the ferment action It is somewhat delayed, however Possibly, the action of the acid is much modified by the protein and salts present But with 6 and 12 mg of acid to the hundred cubic centimeters, with the 500 mg of pancreas powder there is a very different picture In the incubated portion there appears to be a complete destruction of the enzyme, since no change is found in the reaction with starch paste with

the largest amount of ferment solution or after prolonged action on the starch In the portions not incubated, with the acidity the same, starch conversion goes on rapidly in all cases

These experiments show that the amounts of acid used, which are sufficient to destroy the ferment in incubation, are not sufficient to impede the actual digestion

The acid effect is shown in the incubation with the diastase, not in the behavior of the diastase with starch Other experiments showed that starch conversion will go on in presence of much larger weights if acid, but it is evident that very small concentrations of acids destroy the ferment at the incubation temperature

In this connection it may be asked what is the effect of the addition of alkali soon after the addition of acid to the ferment? In the experiments of Panzer, referred to, an attempt was made to answer this question, but the somewhat unfavorable conditions of the experiments made the answer uncertain Some of our experiments on this point suggested a partial recovery of activity, by speedy addition of alkali In mixtures incubated for some time the addition of alkali had but little effect, but when sodium bicarbonate was added soon after the acid, with no incubation, the result was somewhat different, as shown by these experiments

EXPERIMENT 8—Ten c c portions of a 1 10 glycerol extract of pancreas (1 c c of the original strong extract diluted to 10 c c with water) was mixed with 100 mg of salt and acid as below and diluted to 50 c c No preliminary incubation Each portion was then incubated with 50 c c of 1 per cent starch paste for the time shown in Table 5

TABLE 5—DIGESTION OF STARCH WITH PANCREAS EXTRACT MIXED WITH SALT AND HYDROCHLORIC ACID (EXPERIMENT 8)

	Time of Action on Starch, Minutes					
HCl Mg	3	6	10	15	30	90
0	+	+	+	+	+	+
1	+	+	+	+	+	+
3	—	—	—	—	—	—
5	—	—	—	—	—	—
7	—	—	—	—	—	—
9	—	—	—	—	—	—

EXPERIMENT 9—Conditions as in 8 But immediately after mixing with the acid and shaking, the equivalent weights of sodium bicarbonate were added That is, the acid was neutralized as quickly as possible, so that its action on the ferment solution was for a brief time only Then each portion was mixed with the starch paste and incubated as before (Table 6)

TABLE 6—DIGESTION OF STARCH WITH PANCREAS EXTRACT, MIXED WITH SALT AND HYDROCHLORIC ACID, NEUTRALIZED WITH SODIUM BICARBONATE (EXPERIMENT 9)

	Time of Action on Starch, Minutes					
HCl Mg	3	6	10	15	30	60
0	+	+	+	+	+	+
1	+	+	+	+	+	+
3	→+	+	+	+	+	+
5	—	—	→+	→+	→+	→+
7	—	—	—	—	—	—
9	—	—	—	—	—	—

With the weights of acid here used there seems to be a practical destruction of the ferment in a minute or less, of action The failure to convert the starch is not due to the presence of acid, since in Experiment 9 the acid is neutralized before ferment solution and paste are mixed The reaction here is neutral It will be noticed that with the smaller acid concentration there is some little effect by speedy addition of the bicarbonate, but it is not very marked Unless protected by protein or salts the ferment is very rapidly injured or destroyed by traces of acid

There appears, at first sight, to be a discrepancy between the last results and those of Experiment 7, in which 12 mg of acid with ferment diluted to 100 c c, but not incubated, left the diastatic activity still marked In this case, however, the ferment was in the form of 500 mg of a pancreas powder, and the effect of the protein and salts present would doubtless be of moment The glycerol extracts do not contain much protein

From all these experiments it appears that very low concentrations of acid destroy amylopsin Experiment 8 shows that 3 mg of the acid, not neutralized at once, destroys the diastase activity to such an extent that there is practically no starch conversion in ninety minutes This is a strength of 0 006 per cent in the ferment solution, before adding the starch That is, 1 6 c c of 0 1 normal hydrochloric acid to 100 c c destroys even the beginning of starch conversion Kendall and Sherman[8] have shown that about one-fourth of this stops the conversion to the sugar stage in a diastase-starch mixture Our results are not very different from those found by Kjeldahl for the effects of dilute sulphuric acid on malt infusions He found that 10 mg of the acid for 100 c c of paste and infusion practically prevented the starch conversion[9]

Langley and Eves[10] state that 0 005 per cent of hydrochloric acid is destructive to the action of ptyalin, while one-third of this slows the reaction perceptibly, which accords pretty well with our experience

But it must be recognized that the amount of the ferment, or the accompanying proteins, plays a very important part here, as shown by Experiment 4, in which a dilution of the same glycerol extract, but five times as strong as in 2, was employed In that experiment even after incubation, a marked diastatic activity was found in a solution with 18 mg hydrochloric acid to 100 c c of ferment solution This fact may reconcile the somewhat conflicting statements given by Langley and Eves[10] and by Chittenden and Ely[11] for the destructive effect of the acid

8 Kendall and Sherman Jour Am Chem Soc, 1910, xxxii, 1091
9 See Effront Die Diastasen 1900, p 121
10 Langley and Eves Jour Physiology 1883 iv 18
11 Chittenden and Ely Am Chem Jour, 1882, iv, 107

Small amounts of acid are undoubtedly stimulative under certain conditions, and often by the formation of an alkali salt Where relatively large amounts appear to have but slight action, it may be on account of the presence of much protein, which combines with the acid, or on account of salts like bicarbonates or phosphates, which in a sense neutralize it through liberation of a weaker acid of lower dissociation It is certain that the ferment, not protected in some such manner, is extremely sensitive

It is doubtless true that the hydrogen ion content of the solutions in question is the important factor in determining the action on the ferments This has been shown for a number of enzymes and is probably the case here In this connection the papers of Sorenson[12] deserve consideration But in addition to the effect of the hydrogen ions there is also a neutral salt effect which is not so clearly understood and which complicates the general behavior

B THE ACTION OF PEPSIN ON AMYLOPSIN

We come now to the consideration of the behavior of pepsin alone toward the solutions of pancreatic diastase It is commonly held that pepsin is without any action in neutral solutions or solutions of very low acidity, and this is doubtless true in respect to the behavior with coagulated egg albumin and other common protein substances It does not *necessarily* follow that the same should hold for diastase or other enzymes There appear to be no very definite statements on this point in the literature Experiments reported by Wroblewski, Bednarski and Wojczynski appear[13] to lead to the conclusion that a pepsin solution in absence of acid is able to partially destroy malt diastase, and that when acid is present the pepsin increases the action of the latter The diastatic activity is measured by copper suboxid reduced from Fehling solution, but the results of a series of tests are so irregular as to leave one in doubt as to their interpretation We have carried out some tests with glycerol extracts of pancreas and with active commercial preparations from the gland The general method of observation consisted in noting the rate and extent of action on standard starch paste of a series of pancreatic solutions, some of which were not mixed with pepsin solutions, while others received an addition of pepsin solution, in some cases boiled and in others not In such experiments it must be kept in mind that incubation alone materially weakens the strength of amylopsin solutions Observations must be made in sufficient number to measure and allow for this behavior When precautions are taken to have the salt content

12 Sorenson Biochem Ztschr, 1909 xxi and later

13 Wroblewski, Bednarski and Wojczynski Beitr z chem Physiol u Path 1902, i, 229

and other conditions the same it cannot be discovered that pepsin in neutral solution has any effect on the conversion of starch by amylopsin

These necessary conditions are secured here by treating the diastase solution in parallel experiments by active pepsin and pepsin rendered inactive by heat In tests made with the laboratory pancreas extracts and commercial products no definite effect could be proved

C THE ACTION OF PEPSIN PLUS HYDROCHLORIC ACID ON AMYLOPSIN

It has been shown above that even very weak acid has a destructive or inhibiting action on the pancreatic diastase, and that pepsin alone has little or no action on the same ferment It remains to inquire what is the behavior of pepsin *plus* an acid of a strength below the destructive strength on the ferment Will pepsin aided by such an extremely weak acid have any action in excess of that of pepsin alone? It is well known that as far as the action on egg albumin is concerned an acid of appreciable strength is required to bring about digestion, but a weaker acid may be sufficient to exert some effect when pepsin is present If a weak acid retards the action of amylopsin to a certain degree, will the presence of pepsin retard it still more? This is one way to approach the question, and we have carried out a number of experiments in this direction, which are here explained

EXPERIMENT 10.—Five portions of a glycerol extract of pancreas were made up to 100 c c with acid and pepsin as shown in Table 7 These mixtures were incubated through three hours at 40 C and then 1 c c of each was incubated again with 25 c c of 1 per cent starch paste The results are shown in Table 7

TABLE 7.—DIGESTION OF STARCH WITH PANCREAS EXTRACT, PREVIOUSLY INCUBATED WITH HYDROCHLORIC ACID AND PEPSIN (EXPERIMENT 10)

Test No	HCl Mg	Pep Mg	1 Per Cent Sarch, c c	Conversion
1	5	100	25	No conversion
2	5	50	25	42 minutes
3	5	25	25	27 minutes
4	5	—	25	20 minutes
5	—	—	25	11 minutes

The 5 mg of acid in 100 c c equal 0 005 per cent, which amount of acid was found before to have little destructive effect on the amylopsin It may delay the reaction to the colorless end-point, but does not kill the amylopsin The long incubation is more important than the acid in this case With increasing amounts of pepsin we have a check on the rapidity of the conversion, and with 100 mg of pepsin present the reaction becomes so slow as to escape notice

Another set of experiments was arranged in a different way as shown

EXPERIMENT 11.—Ten c c portions of a 1 10 glyceral extract of pancreas were mixed with hydrochloric acid and pepsin to make 25 c c of solution as

shown in Table 8 These mixtures were incubated thirty minutes at 40 C and then, after neutralization, incubated with 25 c c of 1 per cent starch paste The mixtures were tested by the iodin reaction at different intervals

TABLE 8—Digestion of Starch with Pancreas Extract, Previously Incubated (Thirty Minutes) with Hydrochloric Acid and Pepsin and Neutralized (Experiment 11)

	HCl	Pepsin	Incubation with Starch and Result After Minutes				
Test No	Mg	Mg	3	8	13	18	30
1	0 4	10	—	—	—	—	—
2	0 4		→+	+	+	+	+
3	0 8	10	—	—	—	—	—
4	0 8		—	—	→+	→+	→+
5	1 2	10	—	—	—	—	—
6	1 2		—	—	—	—	—

In the pepsin-containing mixtures the conversion of the starch is very much delayed, even with the weaker acid content In absence of pepsin, with the lower acid concentrations, we have a distinct conversion of starch

Experiment 12—Same as 11, but with a preliminary incubation of ten minutes (Table 9)

TABLE 9—Digestion of Starch with Pancreas Extract, Previously Incubated (Ten Minutes) with Hydrochloric Acid and Pepsin and Neutralized (Experiment 12)

	HCl	Pepsin	Incubation with Starch and Result After Minutes				
Test No	Mg	Mg	3	8	13	18	30
1	0 4	10	—	—	—	—	—
2	0 4		+	+	+	+	+
3	0 8	10	—	—	—	—	—
4	0 8		→+	→+	→+	→+	→+
5	1 2	10	—	—	—	—	—
6	1 2		—	—	—	→+	→+

When the mixtures with the pepsin were given more time it was found that a partial change of the starch followed, but slowly There appears to be, therefore, some action of the pepsin and acid which the acid alone does not possess In a later series of experiments the method was varied by adding boiled pepsin solution in the control This introduced the same amount of protein But the results were of the same order With the active pepsin present in the preliminary incubation there was a delay later in the starch digestion

Experiment 13—An active pepsin was used, 100 mg to the 10 c c A solution of an active commercial pancreatin was made containing in 200 c c 1,000 mg of the ferment, 6 mg hydrochloric acid and 200 mg sodium chlorid Fifty c c was incubated with 5 c c of the pepsin solution, boiled, through thirty minutes It was then mixed with 50 c c of 1 per cent starch and incubated until the iodin reaction disappeared *This required twenty-five minutes* A second 50 c c was mixed with 5 c c of active pepsin solution and incubated the same time, and then with starch Forty minutes were now required for the disappearance of the iodin reaction

These various tests show clearly some action of the pepsin on the amylopsin ferment But with commercial specimens the behavior is not always so distinct, possibly because of the varying content of inorganic salts in the latter As the limits of acidity within which amylopsin can act at all are only narrow, it may happen that the salts will neutralize the small amount of acid so that the pepsin in turn cannot be activated This has been the case in some tests made, and here no definite effect in the direction of retardation could be affirmed The subject, therefore, is one which is surrounded with a considerable number of difficulties In the normal action in the body no action of the pepsin on amylopsin is likely because of the regularly occurring neutralization, but in view of the results of the experiments it is possible that some destruction of amylopsin might occur in the stomach It is a question of reaction and excess of acid and the balance between the different phosphates as modifiers of acidity

2 THE ACTION OF HYDROCHLORIC ACID AND PEPSIN ON TRYPSIN

We have here a question much like that discussed under 1 When the action of pepsin on trypsin is referred to it should be understood that pepsin in presence of hydrochloric acid is actually in mind in the great majority of cases It has never been demonstrated that pepsin alone has any action on trypsin The behavior of the acid alone has been frequently studied, but we have no very clear statement as to the behavior of pepsin and the acid under conditions which are important for the present inquiry

Much of our knowledge of the digesting power of trypsin goes back to the time of Kuhne, and many workers in the experimental field as well as on the commercial scale have followed his directions Attempts to produce trypsin by the processes originally described by Kuhne[14] yield at best a very weak product, with which it is difficult to make satisfactory experiments on the behavior of acids and alkalies The effects are in all cases modified by the presence of relatively large amounts of accompanying inert proteins Kuhne spoke of his products as being able to digest bits of fibrin rapidly, but there is nowhere any evidence as to the relative weights of fibrin and ferment employed It is likely that he must have used very much more of crude ferment than of fibrin, unless we accept the somewhat questionable hypothesis of Mays[15] that by accident, almost, Kuhne secured extracts of the beef pancreas which were of a potence not since attainable by others It was not thought necessary at the time of these earlier investigations to add anything to the pan-

14 Kühne Verhandl d naturh med Ver zu Heidelberg 1877, p 194, Untersuch a d physiol Inst d Univ, 1878 1

15 Mays Ztschr f physiol Chem, 1903, xxxviii, 428

creas powder or extract to bring about activation, and this fact has led to some question as to the real activity of the Kuhne products

Whatever their proteolytic power, however Kuhne mentioned that these ferment substances were extremely sensitive to acids He stated that in the presence of 0 05 per cent of hydrochloric acid their activity is still held, but above this destruction soon follows This statement is sometimes misquoted and made to say that in 0 05 per cent acid the ferment is destroyed It was shown by other workers that a much greater acidity is necessary for actual destruction Thus, Engesser asserted[16] that the pancreas powder made by him was able to resist an acidity of 0 5 per cent hydrochloric acid Of the same product Ewald says[17] that in presence of 0 3 per cent hydrochloric acid it is able to dissolve fibrin to the condition of a turbid solution These contradictory statements have long been quoted in the literature It is likely that the last-named writers overlooked the fact that acid of the strength used is sufficient to bring about a partial solution of the fibrin in time In hydrochloric acid of 0 3 per cent strength fibrin swells to a jelly-like mass, and if enough liquid is present, slowly dissolves It is also true that the pancreas powders as made by the Engesser process contained so much protein that they would probably neutralize a considerable amount of acid by combination

It will be recognized that the question of the action of pepsin on trypsin must depend largely on the behavior of the acid itself with trypsin, since pepsin can be used only in acid medium From his experiments Ewald concluded that trypsin is destroyed in acid solutions containing pepsin, and that the rapidity of destruction is proportional to the amount of pepsin present Engesser admitted that any trypsin present as such would be quickly destroyed by acid and pepsin, but he held that in the pancreas powders the trypsin is present as a "zymogen," which has a greater resisting power toward acid and pepsin

These views have mainly a historical interest at the present time, but it is proper to note that views almost as divergent on the two main contentions, the concentration of the acid which destroys trypsin, and the effect of pepsin and acid on trypsin, are still expressed in recent literature Part of the difficulty in the problem seems to be due to the character of the various trypsin preparations employed by different investigators The ferment activity of the pancreas powders made in the laboratory is often very low, and the same is true of most of the market products, as already intimated It is commonly recognized that trypsin products, in general, have but a feeble action on raw egg albumin, but it

16 Engesser Ztschr f klin Med, 1880, II, 192, Maly's Jahresber, 1880, x, 297

17 Ewald Ztschr f klin Med, 1879, I, 615, Maly's Jahresber, 1880, x, 297

is often overlooked that the action on boiled egg albumin is also relatively slow, although easily followed with moderately strong preparations If tested by their action on coagulated egg, many market preparations would have to be pronounced practically inert, while with casein or fibrin a fairly sharp digesting power might be shown, as will be illustrated later The behavior of acid toward trypsin may be best shown by the use of fibrin or casein as a test material, rather than egg

In our efforts to determine the action of weak hydrochloric acid on trypsin we have used a number of products first, a laboratory pancreas powder and a laboratory glycerol extract which had stood long enough to become active, second, samples of Gruebler's trypsin, and finally the products of some American manufacturing laboratories The best of these, by far, is the "trypsin" of Fairchild Brothers and Foster, New York, which may be recommended for scientific investigations Specimens which have come under our observation seem to have about four times the digesting power toward fibrin possessed by the Gruebler preparation, which is often taken as a standard for comparison The "extract of pancreas" of the same firm, F B and F, is also very active, and in the experiments to follow will be often referred to

As in the inquiry on the behavior of acid and pepsin toward amylopsin, we have also here the question of the action of acid alone to consider, and also other points as follows

A The action of water on trypsin

B The action of hydrochloric acid on trypsin

C The action of pepsin and acid on trypsin

In studying the action of water or acid on trypsin we have determined the digesting power of the latter under different conditions, using egg albumin, fibrin and casein for the substratum in different experiments

The egg albumin used for our experiments was prepared in this way A very clear, light-colored, dry hen's egg albumin is brought into this country from China and may be obtained from dealers It seems to be used mainly by bakers, as are other similar products Good samples of this product, which are not hard to secure, dissolve in distilled water to an opalescent liquid, free from lumpy particles and free from odor When this solution is poured into a large volume of boiling distilled water slightly acidified with acetic acid, a finely divided, perfectly white precipitate is secured, as with the solutions from fresh eggs, with this advantage however, that the nitrogen content of the washed coagulum is pretty constant With the very clean albumins which we have been able to secure for this purpose, the nitrogen of the washed coagulum amounts to something over 100 mg for each gram of the original dry

egg With one lot it was practically 106 mg This weight is more nearly constant than may be obtained by using the coagulum from ordinary storage eggs, and serves well for digestion comparisons We have found, however, that the results obtained from some other brands of dry egg albumin were not as satisfactory Much of the American or German "egg" albumin cannot be used at all, as it is not freely soluble

When fibrin is used, it is prepared by thorough washing in water and disintegration in a meat-chopper set to cut very fine The chopped mass is soaked in glycerol until the larger part of the water is removed, which is hastened by pressing out thoroughly and treating with fresh glycerol Finally, the mass is pressed as dry as possible and preserved at a low temperature in bottles containing a small amount of toluene The product may be kept some weeks at a low temperature, and as it is comparatively dry and uniform it may be weighed directly for a series of digestions For one of the last lots prepared in this way, and used for experiments to be quoted, the nitrogen content of 5 gm was found to be 162 9 mg, in the mean, corresponding to about 20 per cent protein It has been noticed, however, that if left in a warm room autolytic digestion of the fibrin takes place rather readily, making the product unfit for use

Casein was employed in the form of a sodium caseinate We used a very pure casein made in the laboratory and dissolved it in weak sodium hydroxid solution, as will be explained below The behavior of water will be studied first

A ACTION OF WATER ON TRYPSIN AT DIFFERENT TEMPERATURES

In pure aqueous solution trypsin becomes inactive much more quickly than pepsin, and markedly so at elevated temperatures In 50 per cent glycerol, trypsin may be kept through months As to the exact effect of elevated temperatures there is a great lack of uniformity in the views of those who have reported on the subject From our experience with a considerable number of trypsin preparations we have come to the conclusion that the salts present are probably the most important factors in modifying the stability

According to the older experiments of Heidenhain,[18] trypsin begins to lose its activity when warmed in aqueous solution to 35 C, but this is denied by Kuhne and others[19] Biernacki[20] states that it becomes inactive in slightly alkaline solution at 45 C Much of the discrepancy is doubtless due to the fact that different observers have worked with

18 Heidenhain Maly's Jahresber, 1875, v, 176, Arch f d ges Physiol (Pflueger's), 1875, x, 557

19 Citation by Oppenheimer, Die Fermente, p 114

20 Biernacki Maly's Jahresber, 1891, xxi, 248

products of very different degrees of purity, but another fact of equally great moment is this, that the various trypsin preparations described in the literature seem to have been extremely weak products, their activities having been measured by the solution of small amounts of fibrin or coagulated egg in digestions carried through many hours

In some experiments made to test this behavior in aqueous solution we have obtained the following results, after using the "extract of pancreas' and the "trypsin" of Fairchild Brothers and Foster, and the glycerol extract of our own production

EXPERIMENT 14.—In Table 10 are given the digestion values obtained by incubating 100 mg of the pancreatin (extract of pancreas) through thirty minutes at 40 C, 50 C and 60 C, in 50 c c of pure water, after which treatment the liquids were brought to 40 C and to each were added 100 mg of sodium carbonate and 3 gm of fibrin (nitrogen content 123 5 mg in mean) The mixtures were incubated again through four hours at 40 C Kjeldahl determinations were made on residue and filtrate

TABLE 10.—DIGESTION VALUES OBTAINED WITH PANCREATIN PREVIOUSLY INCUBATED, FIRST WITH PURE WATER AND SECOND WITH SODIUM CARBONATE AND FIBRIN (EXPERIMENT 14)

	Prelim Digest		Nitrogen, Milligrams		
Test No	Temp C	Fibrin, Gm	Soluble	Residue	Total
1	40	3	103 3	19 3	122 6
2	50	3	77 6	46 5	124 1
3	60	3	19 1	106 0	125 1

It will be seen that the effect of the preliminary digestions at the higher temperatures is to weaken greatly the activity of the pancreatin

In a similar experiment with a preliminary incubation of thirty minutes at 40 C, 25 mg of pepsin were added to the neutral solution, along with the 100 mg of pancreatin At the end of the half hour the mixture was acidified by the addition of 100 mg of hydrochloric acid to the 50 c c Three gm of the same fibrin were added and a new incubation was made at 40 C At the end of forty-five minutes the digestion seemed to be complete On filtering and making Kjeldahl determinations we found

Soluble N	117 5 mg
Residue N	4 6 mg
Total	122 1 mg

Corrections were always made for the nitrogen of the ferments It is evident that under the conditions the pancreatin has not destroyed or appreciably weakened the pepsin activity, as the fibrin digestion is here due to the latter ferment

EXPERIMENT 15.—In a digestion test similar to 14 we used the trypsin, F B F, instead of the pancreatin, 50 mg of trypsin being subjected to the preliminary incubation in 50 c c of water The fibrin used was not from the same lot as the other Final digestion, three hours (Table 11)

TABLE 11.—DIGESTION VALUES OBTAINED WITH TRYPSIN PREVIOUSLY INCUBATED, FIRST WITH PURE WATER AND SECOND WITH SODIUM CARBONATE AND FIBRIN (EXPERIMENT 15)

Test No	Prelim Temp C	Fibrin Gm	Nitrogen, Milligrams		
			Soluble	Residue	Total
1a	40	3	56 0		
1b	40	3	57 9	47 0	104 9
2a	50	3	39 2	65 9	105 1
2b	50	3	38 9	66 1	105 0
3a	60	3	14 0	91 0	105 0
3b	60	3	14 2	91 0	105 2

It will be noted that in this experiment the time of final digestion is three hours in place of four, and that 50 mg of trypsin were used in place of 100 of pancreatin But it is evident that the trypsin has suffered relatively more than the other ferment at all temperatures This seems to be true of the stronger ferments in general As greater activity is secured, it is often at the expense of stability

It has been said that glycerol solutions of trypsin are stable through long periods On diluting such extracts with water we have found that they do not long preserve their activity Even where sterilized water was used for the dilution, we found a marked decrease in action when the solutions stood over night at room temperature The change may be in part due to the comparative absence of inorganic matters in such dilutions At a temperature of 40 C these pure aqueous solutions suffered greatly

Pancreatin solutions (F. B and F as used above), however, when protected with toluene, were found to be fully active after standing forty-one hours at room temperature, as shown by fibrin digestion

In another experiment the effect of longer heating was determined

EXPERIMENT 16.—Fifty-mg portions of trypsin were incubated at 40 C in a volume of 50 c c of water through different periods, as shown below Then to each flask 100 mg of sodium carbonate were added and the incubation continued through three hours at the same temperature with the addition of 3 gm of moist fibrin which had been kept in chloroform water Kjeldahl tests were finally made to determine the amount of digestion (Table 12)

TABLE 12.—DIGESTION VALUES OBTAINED FROM TRYPSIN INCUBATED, FIRST WITH WATER AND SECOND WITH SODIUM CARBONATE AND FIBRIN (EXPERIMENT 16)

Test No	Prelim Digest Minutes	Nitrogen, Milligrams		
		Soluble	Residue	Total
1	30	48 4	49 0	97 4
2	45	50 4		
3	60	53 5	42 6	96 1
4	90	42 6		
5	120	47 5	50 4	97 9
6	180	42 8	54 9	97 7

It appears that at this temperature the effect of incubation is not much greater for a period of three hours than for half an hour Some figures from a later experiment will suggest, however, that the best results are obtained by immediate digestion

In conversation with manufacturers of pancreatins, we have learned that the effects of temperature and solution are recognized It is often insisted that where tests are made on such preparations they should be carried out as quickly as possible after bringing the ferment into solution In such investigations of comparative ferment value it is evident that a temperature of 40 C is the most satisfactory and should not be exceeded

The Effect of the Alkali

As tryptic digestions are always carried out in an alkaline medium, it is desirable to know the action of different amounts of alkali on the ferment A few experiments only will be offered here, as we are concerned with the one alkaline substance only, sodium carbonate It is usually stated that trypsin is not weakened or destroyed by moderate amounts of sodium carbonate, up to about 0 5 per cent This depends, however, on the temperature, as the following tests show While elevation of temperature weakens the trypsin greatly, almost complete destruction follows if much alkali is present In the following experiments portions of 100 mg of pancreatin in a volume of 50 c c were incubated with and without alkali through different times at temperatures of 40, 50 and 60 C They were then brought to the same alkalinity and incubated with 3 grams of fibrin through periods, as shown, at 40 C

EXPERIMENT 17.—Preliminary incubation with and without alkali, using pancreatin (F B and F) and 3 gm of pressed fibrin (N content 123 5 mg)

TABLE 13.—DIGESTION VALUES OBTAINED WITH PANCREATIN PREVIOUSLY INCUBATED WITH AND WITHOUT ALKALI (EXPERIMENT 17)

Test No	Prelim Alkali Mg	Prelim Inc One Hr at C	Alkali Added Mg	Final Inc Hours	Result
1		40	100	6	Digestion completed
2	100	40		6	Digestion completed
3		40 50	100	6	Two thirds digested
4	100	40 50		15	Slight digestion
5		40 50 60	100	15	No digestion
6	100	40 50 60		15	No digestion

In Nos 1 and 2 (Table 13) the time and alkali effects are but slight, as the digestion is complete in six hours In No 3 the action of the higher temperature is shown in the preliminary digestion and when alkali is present at the outset the ferment seems to lose all its activity, practically, as shown in No 4

In Nos 5 and 6 the temperature alone seems to be sufficient to stop digestion These experiments were repeated a number of times with essentially the same results Even when a smaller amount of fibrin was used and the stronger trypsin it was found that the prolonged heating with alkali above the temperature of 40 C resulted in a marked weakening, or practical destruction of the ferment

Finally, the effect of incubation with increasing amounts of carbonate was determined The results show that the ferment is active in presence of a relatively large weight of this alkali

EXPERIMENT 18—Fifty-mg portions of trypsin were incubated during thirty minutes with increasing amounts of carbonate, as shown In three cases enough hydrochloric acid was then added to neutralize all but 100 mg of the carbonate, leaving all of the same alkalinity as the first test In the proper cases sodium chlorid was added to make all alike in this respect, and after the addition of fibrin the incubation was continued through two hours at 40 C Kjeldahl tests were made on the filtrate and residue of the digestion

In two of the numbers 5 and 6 (Table 14), there was no preliminary incubation with the carbonate This was added with the fibrin at the beginning of the actual digestion In the preliminary digestion the volume was 50 c c, and was made to 80 c c in the final digestion in Nos 1, 2, 3 and 4 In 5 and 6 the volume was 50 c c

TABLE 14—DIGESTION VALUES OBTAINED WITH TRYPSIN PREVIOUSLY INCUBATED WITH VARIOUS AMOUNTS OF SODIUM CARBONATE (EXPERIMENT 18)

Test No	Mg Prelim Alk	Mg Final Alk	Soluble Nitrogen, Milligrams	Residue Nitrogen, Milligrams	Total Nitrogen, Milligrams
1	100	100	77 1	22 7	99 8
2	200	100	79 4	22 1	101 6
3	300	100	84 6	16 0	100 6
4	500	100	85 7	16 0	101 7
5		100	98 0	4 5	102 5
6		500	98 0	4 2	102 2

The fact is brought out pretty clearly that in the preliminary incubation, the presence of the carbonate is protective of the ferment It is also shown that where there is no preliminary incubation the final digestion is much nearer complete Part of the apparent effect may, however, be due to the salt added, or formed in the other cases The important fact which is shown is that the trypsin ferment exhibits the same activity in the presence of 1 per cent of sodium carbonate that it exhibits with 0 2 per cent In practice digestions may be carried out within these limits with like results, as long as the temperature is no higher than here

B THE ACTION OF ACID ON TRYPSIN

As our object was mainly a practical one, to determine the possible part played by pepsin in aiding the acid destruction of trypsin, we have confined ourselves to a study of the behavior of hydrochloric acid To learn the effect of pepsin on trypsin it is, of course, necessary to fix first the action of the acid with which the pepsin works and for this purpose hydrochloric acid alone comes practically into consideration Trials have been made with both egg albumin and fibrin, and we shall quote

first a few of the egg-digestion experiments to show an important point regarding the behavior of this form of protein The sodium carbonate mentioned was always taken as anhydrous In all cases a correction was made for the nitrogen of the ferment itself

EXPERIMENT 19.—Fifty mg of F B and F trypsin plus coagulum from 1 gm of egg (106 mg nitrogen) plus 50 mg of sodium carbonate in volume of 50 c c Digested ten hours at 40 C The undissolved egg was filtered off

Soluble N = 25 5 mg

EXPERIMENT 20.—Fifty mg of same trypsin plus 30 mg of hydrochloric acid made to 50 c c with water and incubated one hour at 40 C Then 100 mg of sodium carbonate was added with the coagulum from 1 gm of egg and the digestion continued through ten hours at the same temperature

Soluble N = 27 2 mg

EXPERIMENT 21.—Fifty mg of same trypsin, plus coagulum from 1 gm of egg, plus 200 mg of sodium carbonate made to volume of 50 c c and digested ten hours at 40 C

Soluble N = 14 8 mg

It is apparent from these tests that egg albumin is but slowly digested by trypsin This fact has been occasionally referred to in the literature, but it is not clearly recognized Some observers have actually recommended the use of uncoagulated egg white for such digestion tests to determine the value of trypsin, but it is absolutely unsuitable for the purpose, as will be pointed out below The fibrin digestion is much more rapid, as a few experiments will indicate

EXPERIMENT 22.—Fifty mg of same trypsin, plus 100 mg of sodium carbonate, plus 3 gm of fibrin (97 7 mg of nitrogen) made to 50 c c, and digested at 40 C The fibrin was all dissolved in thirty minutes

EXPERIMENT 23.—Fifty mg of Gruebler's trypsin, plus 100 mg of sodium carbonate, plus 3 gm of the same fibrin, made to 50 c c volume and digested at 40 C Over two hours required for complete solution

The last experiments show the marked difference between the two kinds of trypsin, which results were duplicated by many other observations, but they show also the ease of fibrin digestion as compared with that of egg In one of the egg experiments a small amount of hydrochloric acid was employed in a preliminary digestion with the trypsin This amount of acid did not appear to have any inhibiting effect as far as the subsequent digestion, after neutralization, was concerned, using either egg or fibrin With such strengths of acid in preliminary digestion the solution of the egg was always but partial, as was the case when no acid was used But the fibrin digestion could easily be brought to completion after such treatment It was found that a still greater degree of acidity in the preliminary incubation seemed to have but a slight action on the trypsin, when tested with fibrin, and this was shown quantitatively with the F B and F ferment in the following tests

EXPERIMENT 24.—A series of solutions was made containing in 50 c c 50 mg of the trypsin and increasing weights of the acid up to 100 mg These solutions were incubated thirty minutes at 40 C and then mixed with enough sodium carbonate to neutralize the acid and leave 100 mg of the carbonate present in each case Enough sodium chlorid was added to four of the mixtures to make the content the same as in the mixture with the greatest acid originally Finally 5 gm of fibrin, containing 162 8 mg of nitrogen, was added to each mixture and all were incubated five hours at 40 C The rate of digestion appeared to be nearly uniform At the end of the period all of the solutions were filtered and Kjeldahl determinations made on filtrate and residue The results were as shown in Table 15

TABLE 15.—DIGESTION VALUES OBTAINED WITH TRYPSIN PREVIOUSLY INCUBATED WITH ACID, THEN MIXED WITH SODIUM CARBONATE (EXPERIMENT 24)

			Nitrogen, Milligrams		
Test No	HCl Mg	Trypsin Mg	Soluble	Residue	Total
1	60	50	156 6	7 8	164 4
2	70	50	152 3	11 5	163 8
3	80	50	151 0	11 9	162 9
4	90	50	151 4	13 4	164 8
5	100	50	151 4	10 6	162 0

It will be seen that a preliminary digestion in a medium containing 0 2 per cent of hydrochloric acid does not destroy or practically retard the activity of the trypsin The digestion, after neutralization, is as complete in the mixtures which contained the largest amount of acid as it is in the one with the smallest amount The weights of total nitrogen recovered are not quite uniform This is doubtless due to the fact that absolute uniformity in the nitrogen content of the moist fibrin could not be secured.

EXPERIMENT 25.—The preceding experiment was repeated, using a new and much dryer preparation of fibrin Three-gram samples contained, in the mean, 168 8 mg of nitrogen In these tests the acid added in the preliminary incubation was greater, as shown in Table 16

TABLE 16.—DIGESTION VALUES OBTAINED AS IN TABLE 15, EXCEPT FOR USE OF A DRYER FIBRIN AND LARGER AMOUNT OF ACID (EXPERIMENT 25)

			Nitrogen, Milligrams		
Test No	HCl Mg	Trypsin Mg	Soluble	Residue	Total
1	100*	50	18 2	154 0	172 2
2	120	50	129 3	37 5	166 8
3	140	50	139 4	25 8	165 2
4	160	50	130 7	43 5	174 2

* + 25 pepsin

In the first of these tests the preliminary incubation of thirty minutes with pepsin, in addition to the acid present, resulted in a marked lowering of the efficiency of the trypsin in the final fibrin digestion In the other cases the acid digestion alone, with amounts of acid reaching 0 32 per cent, shows a slight impairment only of the activity of the trypsin For our purpose it was not thought necessary to carry the acidity beyond this last value, or to increase the time of preliminary

incubation beyond thirty minutes We have kept in mind the practical bearing of these questions on problems connected with actual conditions in the human body

It is evident from the preceding tests that trypsin, even when not protected by a great excess of protein, is able to withstand an acidity of over 0 3 per cent of hydrochloric acid through thirty minutes at 40 C Somewhat similar results have recently been reported by others, to be referred to, but the weights of protein used in the final tests of the residual trypsin activity have usually been so small as to leave the question really very much in doubt It will be recognized that in these experiments we are not dealing with the matter of the digestion by means of trypsin in acid solution It has been shown by many observers that in presence of acid the activity of trypsin is practically nil [21] We are simply concerned with the effect of acid on trypsin in absence of much protein

In this connection it is in order to refer to some similar experiments carried out with casein as the substratum Casein may be made in very pure dry form and serves as an excellent standard substance We have used it in different ways and in this series of tests by the method of Fuld,[22] according to which a weak sodium caseinate is prepared and treated with the ferment until it no longer gives a precipitate with dilute acetic acid Ordinarily the method has been worked with very small weights of casein, usually 4 mg We found a larger weight preferable as a substratum, as it permits the use of stronger ferment solutions One set of experiments may be quoted as illustrating the whole series of tests

Experiment 26.—Our casein solution was made with the minimum amount of alkali required for the formation of the acid salt [23] As a trypsin we used in this series the "Extract of Pancreas" of Fairchild Brothers and Foster, already referred to Six test tubes were charged with amounts of the pancreatin, as shown below, and to each was added 5 c c of 0 2 per cent hydrochloric acid All the tubes were incubated one half hour at 40 C, then neutralized exactly To each tube 6 c c of 0 2 per cent casein solution was added and these, in turn, were incubated one hour and then the tests for unaltered casein were made by adding to each tube three drops of the acetic acid reagent (1 part of acid, 50 parts of alcohol, 49 parts of water) Table 17 shows the relations It is seen that even with the weakest pancreatin concentration, and after the half-hour incubation with acid, the casein is completely changed in the final digestion

21 See, for example, Chittenden and Cummins Am Chem Jour, 1885, vii, 49

22 Verhandl d Ver inn Med, 1907, Maly's Jahresber, 1907, xxxvii, 446, 1908, xxxviii, 375

23 Long Some Investigations on Salts of Casein, Jour Am Chem Soc, 1906, xxviii, 372

TABLE 17.—DIGESTION VALUES OBTAINED WITH TRYPSIN TESTED AGAINST CASEIN (EXPERIMENT 26)

Test No	Pancreas Ext., Mg	Casein, Mg	Result
1	2 0	12	Complete digestion
2	1 3	12	Complete digestion
3	0 8	12	Complete digestion
4	0 5	12	Complete digestion
5	0 3	12	Complete digestion
6	0 0	12	No apparent change

The results of this method fully confirm those previously obtained by the fibrin digestion

C THE ACTION OF ACID AND PEPSIN ON TRYPSIN

Having discussed the action of weak hydrochloric acid on trypsin, we now come to the practical question of the behavior of pepsin and acid working together As shown above, we are concerned with hydrochloric acid only, as acids of lower ionization do not come into the question at all In fact, it is essentially with the hydrogen concentration from this acid that we have to deal, and our experiments have been so arranged as to secure the concentration indicated by the percentage weights used To this end we have used with the acid only small weights of strong ferments and no protecting protein to combine with the acid With the addition of the pepsin in the preliminary incubation the same plan will be followed

At the outset it was intimated that the few investigations which have been carried out to shed light on this subject have not furnished very concordant or satisfactory results Some of them have been roughly qualitative only, not quantitative Much of the trouble has been with the weak trypsin products employed in the tests, making it necessary to incubate through relatively long periods, and thus introduce the disturbing time and temperature factor The weak ferments, with high protein content, combine themselves with so much acid that a further disturbing factor is introduced It is practically useless to employ the ordinary "pancreatins" of the market in such investigations, as their trypsin content is always low, and often nil

Among the more recent investigations in this direction which may be quoted, two will serve as illustrations

Some extended observations have been published by Wroblewski, Bednarski and Wojczynski[24] on the behavior of ferments toward each other and toward acids and alkalies Some of their tests were carried out with raw egg white as substratum and others suffer from other errors of method or manipulation, so that many of the results are quite meaningless In some of their experiments the weights of ferment and egg

24 Wroblewski, Bednarski and Wojczynski Beitr z chem Phys u Path 1902, 1, 288

periods the digestion of the casein was found to be practically complete, suggesting a weakening, or partial destruction, rather than a complete destruction of the trypsin by the pepsin

Following these tests a considerable number of others were made in a different way In some of these the coagulum from the solution of dried egg albumin was used and in this manner

EXPERIMENT 29.—Fifty mg of trypsin, 30 mg of hydrochloric acid, 50 mg of pepsin in boiled solution and the coagulum from 1 gm of egg were made to 50 c c and incubated one hour at 40 C The mixture was then neutralized with 100 mg of sodium carbonate and incubated through ten hours

Soluble N, corrected for N of ferments = 36 4 mg

EXPERIMENT 30.—Same as last with this exception the pepsin solution added was not boiled

Corrected soluble N = 26 9 mg

The acid content is here so low that little or no peptic digestion of the egg might be expected The digestion is probably wholly due to the trypsin, which seems to be weakened to some extent by the pepsin in the second experiment

EXPERIMENT 31.—In a series of flasks 50 mg of trypsin, 25 mg of pepsin (both F B F) and increasing amounts of hydrochloric acid were made to a volume of 50 c c The flasks were incubated at a temperature of 40 C through thirty minutes and neutralized with enough sodium carbonate to leave an alkalinity of 0 2 per cent of the carbonate Salt was added to four of the flasks to make the content the same as that in the one with the highest original amount of acid To each one 5 gm of fibrin was added (mean nitrogen content 160 1 mg) and a further incubation of six hours was made The amounts of soluble nitrogen were determined as in Table 18

TABLE 18.—DIGESTION VALUES OF TRYPSIN PREVIOUSLY INCUBATED WITH PEPSIN AND HYDROCHLORIC ACID, THEN NEUTRALIZED (EXPERIMENT 31)

Test No	HCl, Mg	Soluble N, Mg
1	25	9 25
2	30	17 57
3	35	7 77
4	40	3 33
5	50	6 79

Controls without the pepsin showed complete digestion of the fibrin

There appears to be here a marked destruction of the trypsin In another set a new fibrin was used with mean nitrogen content for 5 gm = 167 4 mg This was incubated with the pepsin and trypsin in some cases, and in others was added after the preliminary incubation, as shown below

EXPERIMENT 32.—In a number of flasks different weights of trypsin and pepsin (as above) and acid were made up to 50 c c and incubated thirty minutes at 40 C In 1 to 6 (Table 19) the fibrin, 5 gm, was added before the incubation, and in 7, 8 and 9 after the incubation and neutralization In all cases enough sodium carbonate was added to make the final alkalinity equal 0 2 per cent The period of incubation after adding the alkali was four and one half hours

TABLE 19—DIGESTION VALUES OF TRYPSIN INCUBATED AND NEUTRALIZED BEFORE AND SOME AFTER ADDITION OF FIBRIN

No Test	Trypsin Mg	Pepsin Mg	HCl Mg	Fibrin Gm	Nitrogen, Milligrams Soluble	Residue
1	100	25*	35	5	159 6	7 6
2	100		35	5	157 9	8 1
3	100	25	35	5	159 9	6 3
4	100*	25	35	5	88 4	74 8
5		25	35	5	105 7	60 3
6				5		167 4
7	50		40	5	lost	5 2
8	50		50	5	157 8	10 3
9	100	25	35	5	42 9	122 6

* The ferment was added in a boiled solution

Nos 1 and 2 of Table 19 show the digestive action of trypsin alone, as in one case no pepsin is present and in the other killed pepsin We have a nearly complete solution of the fibrin No 3 shows practically the same result, and apparently no destruction of trypsin because this is protected by the fibrin present There is also some digestion by the pepsin in acid solution, which obscures the effect of the trypsin to some extent This is a mixed digestion In No 4 we have the effect of the pepsin and acid alone, as the accompanying trypsin was boiled No 5 shows a somewhat similar condition, indicating that the pepsin alone can digest but a part, less than two-thirds of the fibrin No 6 shows the nitrogen content of the fibrin In Nos 7 and 8 we have this difference in condition, that the fibrin was added after the preliminary acid diggestion, with no pepsin present The trypsin remained active enough to digest the fibrin finally In No 9 trypsin and pepsin are digested together without protecting fibrin When the latter is added later the trypsin seems to be now too weak to digest much of it This coincides with some of the earlier tests in indicating, under these conditions, a partial destruction of trypsin by pepsin

A much more complete destruction of trypsin is shown if the digestion in presence of acid is continued beyond an hour As long as the temperature of digestion does not go above about 40 C, the effect of incubation in water alone is not noticeable for several hours, with most trypsins, but there seems to be some difference in products from different sources in this respect, due possibly to salt content In weak acid, up to 0 2 per cent hydrochloric acid, the destruction of the trypsin is not rapid

A large number of experiments have been made to show the effect of acid and pepsin when the preliminary incubation is prolonged In these tests the coagulum from 1 gm of dry egg, prepared as explained above, was employed to measure the action The mean nitrogen content of a gram was 106 mg A few of the results from such experiments are sufficient to quote, as those from the use of larger amounts of acid or from longer incubation show, of course, the same destructive action

EXPERIMENT 33—In a number of flasks hydrochloric acid, pepsin and trypsin, in amounts shown below, were made up to a volume of 50 c c and incubated through two hours at 40 C In some of the tests the trypsin was added in boiled solution Then sodium carbonate was added to neutralize the acid and leave

0 2 per cent, more trypsin added, boiled or unboiled, and the coagulum from 1 gm of egg A new incubation was carried out through four hours, when the amount of soluble nitrogen was determined A correction was made for the nitrogen of the ferments added This is assumed to be all soluble

TABLE 20 —Digestion Values of Trypsin After Prolonged Incubation with Pepsin and Acid (Experiment 33)

No Test	Pepsin Mg	Trypsin Mg	HCl Mg	Trypsin After Incubation Mg	Nitrogen, Soluble,	Milligrams Residue
1a	25	100	20	100*		108 4
1b	25	100	20	100*		106 7
1c	25	100*	20	100	95 4	16 2
1d	25	100*	20	100	90 4	18 5
2a	25	200	20	200*		108 6
2b	25	200	20	200*		108 9
2c	25	200*	20	200	62 0	44 1
2d	25	200*	20	200	†	†
3a	25	100	50	100*		103 9
3b	25	100	50	100*		109 5
3c	25	100*	50	100	71 1	28 3
3d	25	100*	50	100	68 9	30 1

* Trypsin in boiled solution
† Lost

The trypsin used in the experiments Nos 2a to 2d was a much weaker product than that used in the other cases

These experiments show that the trypsin freshly added digests the egg very well, but after contact with acid and pepsin through two hours it is inert We have found that some samples of trypsin or pancreatin heated with the acid of the strengths here used become inert, even when no pepsin is used But the process of manufacture leaves some of these products with a larger amount of inorganic salts present Three well-known brands of pancreatin of American manufacture show 6 7, 7 6 and 14 8 per cent of ash, respectively These facts must be kept in mind when comparisons of such products are made

It is evident from the sum of the experiments quoted that, *in vitro,* the destructive action of acid on trypsin is much accelerated by the presence of pepsin This action is modified to some extent by excess of protein present, and it is this fact which makes a definite statement as to what happens in the body somewhat difficult An accumulation of acid beyond that necessary to combine with protein to form acid albumin would undoubtedly interfere with the activity of trypsin present at the same time There appears to be no recovery of trypsin after neutralization of the inhibiting acid In the actual conditions in the stomach the acid ordinarily accumulates beyond the protein-saturating point and would then be able to work on trypsin ingested These experiments appear to uphold the view held by many physiologists that under practical conditions pepsin is antagonistic to the tryptic ferment

In the course of our studies of these ferments we have had occasion to examine most of the pancreatins of American manufacture There are numerous brands on the market, but many of them come from the same source It has been found that while some of these have a fair amylolytic action, their tryptic power is usually low Some are, indeed, quite inert This must follow from the careless process of manufacture often followed, which may consist in the rapid drying of the pancreas gland, the removal of most of the fat by extraction with some convenient solvent and final grinding to powder The temperature of drying undoubtedly is high enough to destroy the sensitive ferments. The low wholesale price at which some of these powders are sold would point to a very hasty process of manufacture The tests prescribed by the Pharmacopeia for pancreatin do not encourage a higher strength than that usually found The standard is low enough to suit the indifferent manufacturer In this respect there is a curious contrast between the treatment of pepsin and pancreatin which certainly calls for correction.

These criticisms hold for the edition of the Pharmacopeia now in use, and they seem to hold for the revision of the work soon to appear The conversion of 25 parts of starch, made into paste, to the colorless endpoint by 1 part of ferment, in five minutes, is a requirement too low to have much practical value It is not a fair standard, since there are a number of commercial pancreas products known which have a far greater degree of activity The Pharmacopeia describes pancreatin as containing the other ferments of the pancreas, as well as the amylopsin Why is no proteolytic standard adopted? The market grades will remain weak as long as the Pharmacopeia is silent on this point Possibly all concerned recognize the seeming futility in making a product the strength of which is almost certain to be impaired, and perhaps destroyed, in passing through the stomach

This point should be kept clearly in mind by physicians that the pancreatins of the market are mostly weak, and further, that if they were strong there is no certainty that they would be of much more use in actual practice, as commonly administered The question may well be asked Except for the purpose of curdling and partly predigesting milk, why should pancreatins be used at all? As digestive agents their usefulness is certainly of a very low order, and it is likely that there would be no practical loss in dropping pancreatin entirely from the Pharmacopeia

As illustrating the actual strength of the best known brands in this country, the following table is given The samples tested were collected fresh from the manufacturers in January, 1913, and examined at once as to their behavior with starch and fibrin A year later, January, 1914, they were tested again It has been repeatedly pointed out that the

presence of certain inorganic substances is essential for the development of the maximum activity of the amylopsin In many cases the process of manufacture leaves enough of this in the finished product usually chlorid and phosphate, but occasionally this is not the fact and more salt should be added by the analyst The Pharmacopeia fails in this very important provision, and the failure may sometimes lead to an improper rating of a product examined

The starch-digesting power of the pancreatins will be given first, the different products being designated by number rather than by name The numerical values given show the number of minutes required to convert 500 parts of starch to the colorless end-point by 20 parts of the ferment, that is, the low standard of the Pharmacopeia is taken for comparison The amount of salt added was sufficient to insure a concentration of 0 1 per cent

TABLE 21.—STARCH DIGESTING POWER OF PANCREATIN

	Tests Made in 1913		Tests Made in 1914	
Number	No Salt Added	Salt Added	No Salt Added	Salt Added
1	3	3		3
2	15	5	105	27
3	5	4	15	10
4	60	15		10
5	5	4		14
6	4	4		5
7	10	4	*	80
8	10	4		25
9	5	4		7
10	5	4		4
11	†			
12	10	4		
13	15	4		

* Inert

† Low in amylopsin

It will be seen that, when perfectly fresh, all of these samples, except one, satisfy the requirement of the Pharmacopeia, as long as sufficient salt is present In absence of added salt half of the samples are found to be weak by this very lenient test One year later over half of the samples are found to be weak, even with the salt, and some had become practically inert Three samples retained their former value, and these were characterized by a high original content of inorganic matters This is evidently a factor in the keeping quality as well as in the residual activity It may be recalled that there are pancreas products on the market which are not sold under the name of pancreatin and which have four times the starch-converting power here shown It

is therefore evident that in actual digesting value and in keeping qualities some of these products are far from satisfactory

We find a somewhat similar situation when we come to the consideration of the proteolytic power, as measured by the action on moist fibrin In each test, in the first examination, 25 mg of ferment, 2 gm of fibrin and 50 c c of 0 2 per cent sodium carbonate solution were incubated at 40 C until the digestion was practically complete, as shown by the disappearance of all but stray flakes of the fibrin In the tests made in 1914, 100 mg of the ferment were taken in each case because the products had become weaker

TABLE 22—Proteolytic Power of Pancreatins

No	1913 Tests	1914 Tests			
	Digestion Time	Change in One Hour	Change in Three Hours	Primary Albumose	Secondary Albumose
1	80 minutes	Very slight	Slight	Slight	Trace
2	Apparently no change	Very slight	Very slight	None	None
3	120 minutes	Very slight	Very slight	Trace	Trace
4	720 minutes	Very slight	Very slight	Trace	None
5	100 minutes	Very slight	Very slight	Trace	None
6	40 minutes	Slight	Marked	Some	None
7	100 minutes	Slight	Marked	Some	Trace
8	Very little change	Very slight	Very slight	Trace	None
9	40 minutes	Slight	Slight	Trace	Trace
10	12 minutes	Nearly all	All gone	Large	Large
11	8 minutes	All gone	All gone	Large	Large
12	70 minutes				
13	105 minutes				

The terms primary and secondary albumose are here used in the old sense, the first denoting the precipitation by half saturation with ammonium sulphate, while the notation under secondary albumose indicates the result by completely saturating the filtrate from the primary albumose with the sulphate The results are mostly trifling in amount

In examples 10 and 11, Table 22, we see what may be expected from a really active tryptic preparation but in most of the other cases the amounts of fibrin digested by the year-old specimens are very low, and probably so low as to merit no consideration Even when fresh, most of the pancreatins are slow in their tryptic action and two are inert The manufacturers of pancreatin have not yet learned the secret of making a product with a reasonable degree of tryptic activity, if this is indeed considered worth while in view of the probable partial destruction the stomach

It is evident that samples of pancreatin which have stood on drug-store shelves a year or more are liable to be very low in tryptic as well as in starch-digesting power The question naturally presents itself, What, then, is the practical value of these preparations? Certainly, pancreatin, as now made, leaves much to be desired It has yet to earn a place in the category of remedies which are actually useful

Northwestern University Medical School

MAY 16 1914

The Archives of Internal Medicine

Vol XIII APRIL, 1914 No 4

SYSTEMIC BLASTOMYCOSIS

A REPORT OF ITS PATHOLOGICAL, BACTERIOLOGICAL AND CLINICAL FEATURES [1]

A M STOBER, M D
CHICAGO

The object of this report is so to relate the observations made in connection with a number of cases of systemic blastomycosis that a better understanding may obtain of the disease not only in this locality, where clinicians are already quite familiar with it, but also in places where it has been encountered but rarely or not at all

Two summaries of the literature have already been made, that by Hektoen[1] dealing with thirteen cases, and the one by Montgomery and Ormsby[2] with twenty-two

The second of these reviews contains brief accounts of the illnesses of four patients in this series and the post-mortem examinations on the bodies of two of them The other two patients have since died and we are able to add the more detailed clinical records and the final results of the studies of material from two additional post-mortem examinations

[1] Under the title "Medical and Surgical Reports of the Cook County Hospital for the Six Months Ending July 1, 1890," the first report containing scientific articles from this institution was published, it was edited by Louis J Mitchell, M D A second report also edited by Dr Mitchell with a similar title covered the succeeding six months The third scientific report was entitled "Cook County Hospital Reports, 1906" It was compiled and edited by a committee of the attending staff and contained material which for the most part had been published elsewhere The fourth and last report of this character was published in 1910 Like the third it bore the title "Cook County Hospital Reports" It was edited by a committee with Dr E R LeCount as chairman None of the articles in it had or have since been published elsewhere Fifteen hundred copies of this report were printed bound in black cloth Only three are at present known to be in existence The remainder disappeared about the time there was a change in the board of commissioners of Cook County as well as a considerable change in the political composition of this board What became of these reports is not definitely known According to rumors they were burned by mistake It has been thought best to reproduce the articles on blastomycosis which occupy 160 of the 196 pages in the original volume, since the observations they contain regarding this relatively rare disease would otherwise be lost or escape notice entirely

1 Hektoen, Ludwig Systemic Blastomycosis and Coccidioidal Granuloma, Journ Am Med Assn 1907, xlix 1071 Chrurgische Festschrift

2 Montgomery F H, and Ormsby, O S Systemic Blastomycosis, THE ARCHIVES INT MED, 1908, ii 1

On account of the involvement of the bones, Dr E W Ryerson[3] has considered the clinical and pathological relations of such changes to diseases for which it might be mistaken and utilized the observations made in connection with two cases of this series To these five cases which have been but partially reported, there are added the hitherto unpublished observations on six additional cases, making the total number reported twenty-nine I know of seven other instances of systemic blastomycosis, some of which will undoubtedly be published in the near future, six of these concern patients in Chicago or the immediately surrounding country Of the twenty-nine cases reported, fourteen have concerned patients in Cook County Hospital, and of these eleven have occurred in the last two years The total, thirty-six, represents an increase of over 200 per cent during the last two years, and this without doubt is due to the great familiarity of physicians, especially the better clinicians, with the manifestations of the disease

There are good reasons, as others have pointed out, for believing that the disease is frequently mistaken for tuberculosis, and judging from a number of the clinical histories in this series, it is evident that such errors are still likely to occur even here where the condition is perhaps best known

The disease seems to be one of early life, as two-thirds of the patients have been under the age of 34, the youngest 17, the oldest 58 years of age, all but two occurred in men The occupation in nearly every instance was one involving hard manual labor and considerable exposure Heredity seems to have no influence, and no definite instance of contagion has been established When this seems to have occurred, the exposure of all affected to a common case must be remembered The majority of the patients have been foreigners, especially Poles and Italians, but no special nativity predilection has been noticed Nearly all were poor and lived in unhygienic environment Injury seems to have been a predisposing factor in several instances, either by affording opportunity for direct inoculation (see Cases 3 and 12 in the accompanying bibliography) or producing a region of lowered resistance for the development of the infection already existent (Cases 8 and 23)

The cause of this disease is now generally recognized to be a mold which, because of the budding form of the organism in the tissues, has been called blastomyces

The reasons for considering the blastomyces as the cause of blastomycosis are briefly, its presence in all the new or recent lesions, the readiness with which it is obtained from such lesions in pure culture, and the experiments on animals which demonstrate its pathogenicity The organism has been recovered from the blood and is present in the pus of

3 Ryerson, E W Am Jour Orthop Surg, 1908, vi, No 1

unopened and discharging abscesses, quite regularly present in the sputum and sometimes demonstrated in the urine or feces

Numerous descriptions of the organism, its cultural peculiarities and variations in morphology have been published [4]

Some confusion still exists regarding the classification of blastomycetes and especially in regard to the significance of variations observed during the cultivation of different strains Differences were first noticed in the study of organisms obtained from the cutaneous lesions of blastomycotic dermatitis, and Ricketts, in comparing the characteristics of seventeen organisms, six under his personal observation, found variations in their manner of growth sufficiently constant and distinct to allow of a classification into three morphological types, namely, blastomycetoid, oidium-like and hyphomycetoid

The identity of the organism causing these cutaneous lesions and those found in the systemic infections was at first questioned, but as cases of the latter became more numerous, their identity was generally recognized It was then ascertained that the variations were not sufficiently constant to allow of a separation into distinct types Hamburger [4] in a recent comparative study of four strains of blastomycetes obtained from patients with systemic infection, found one which differed somewhat from the other three, but not sufficiently to prevent the conclusion that the organisms were identical Still more recently Montgomery and Ormsby have asserted that all the morphological types hitherto described have been observed at different times in the same strain Many of the studies dealing with these matters have extended over rather limited periods of time and included comparatively few strains We have studied altogether eleven strains eight from the cases here reported (Cases 19 to 26) and three others, to the source of which reference will be made The observations extended over a period varying in the individual strains from two to twenty months In all instances the organisms were obtained in pure culture from the pus aspirated from abscesses, and similar organisms were also obtained in cultures from the sputum in most cases They were also obtained from the blood of one patient (Case 19) and from the eye, pericardium and pleura in another (Case 20) A number of different cultures as to source were therefore obtained from each patient, and as they possessed no differences (except in Case 19) only one or two from each patient were used in the comparative study [5]

4 Ricketts, H T Jour Med Research, 1901, vi, No 3, Montgomery, F H A Brief Summary of the Clinical, Pathologic and Bacteriologic Features of Cutaneous Blastomycosis (Blastomycetic Dermatitis of Gilchrist), Jour Am Med Assn, 1902, xxxviii 1487, Hamburger, W M, Jour Infect Dis, 1907, iv, 201

5 The organisms will be designated according to the case from which they were obtained

As may be presumed, the morphological and cultural details of these different strains of organisms presented marked similarities, and in order to prevent repetition of details one general description will be given with the variations noted

The organisms in the tissues discharges and pus corresponded with those described in previous reports They appeared in pairs or clumps as round or oval bodies, and budding was a common feature (Fig 1) [6] The size usually varied, those about 20 microns in diameter being the most frequent, although larger forms (from 25 to 30 microns) were not uncommon, and innumerable smaller forms (from 3 to 5 microns)



Fig 1—Specimens of pus containing blastomycetes mounted in a solution of potassium hydroxid Note the round and budding forms, the refractile capsule and the vacuoles and granules in the center (Courtesy of Dr O S Ormsby)

were sometimes found Ordinarily the outer refractile capsule and clear central portions could be recognized The capsule was homogeneous and double-contoured and varied in thickness, being thinner in the smaller and younger forms It was occasionally absent, especially in the organ-

6 The illustrations presented in this and the following articles on blastomycosis were (except where noted) prepared by me and formed the basis of a research exhibit presented at the 1908 session of the American Medical Association The roentgenograms were prepared by Dr H E Potter roentgenographer to the Cook County Hospital and are included in an article on "Radiographic Appearances of Pulmonary and Osseous Blastomycosis," published in the American Quarterly of Roentgenology May 1910

isms of old foci In sections of a bone-abscess a thick or adventitious capsule described by other writers was found The capsule rarely stained by the ordinary methods The central portion in the fresh condition was usually clear, and stained more or less granular, taking basic stains and coloring light blue with hematoxylin, and red or violet with polychrome methylene-blue Occasionally the central portion stained intensely, and when the capsule was absent or indistinct, such organisms resembled small lymphocytes In other organisms it remained unstained or presented the same color as the surrounding tissue In the fixed and stained as well as the unstained organisms, one or more vacuoles may be present, but the most common feature in the former was the presence of small spherical basophilic granules, usually from three to ten in number Although quite regularly arranged, their distribution in the cell cannot be said to be characteristic They were often found in the budding forms and in some instances appeared to be contained entirely in the bud The association of these granules with spore-formation has been suggested by a number of writers on this subject According to most observers proliferation in tissue occurs by budding Hyphae have not been found in the tissues, and sporulation has been suggested in but one instance (Case 8), the extremely small size of the organisms and occurrence in clusters being the chief reason given In old cultures a vacuolization occurs which strongly resembles sporulation [7]

The blastomycetes grow readily on ordinary mediums No difficulty was experienced in obtaining growths on sterile moist bread, paper, cardboard, cotton, sawdust, fruit and vegetables Isolation from other organisms can usually be accomplished by making a thin stroke on a dry agar surface, the tendency of the blastomycetes to extend over the surface

7 In our studies, a process thought to be endosporulation was observed in one instance In an agar-slant culture of a strain obtained from Case 19, sixteen months old at the time, a contamination occurred and the surface became covered with a slimy saprophytic growth Examination of a smear before discarding disclosed the presence of a large number of round forms of blastomycetes scattered among the bacteria, which belong to the *Bacillus subtilis* group (Fig 2) Budding and hyphal forms were absent Bodies considered to be spores appeared in the organism as round clear objects which varied markedly in size and distribution (Fig 3) Sometimes only one or two were present in a cell, but they usually occurred in large numbers, irregular in size In some cases a fairly regular distribution was noted The larger forms sometimes extruded from the cell and in other instances the cell outline was lost in a zooglea like mass of cluster of the smaller forms, similar to those observed in tissues That these appearances were due to a reproductive rather than an involution process was assumed because of the innumerable small round forms present, the similarity, even to the refractile capsule, of the younger forms within the parent cell to those living free in the neighborhood, and the absence of any other form of reproduction The same process also was observed to a less extent in a subculture from the tube described above It was impossible to isolate or obtain growths of the blastomycotic organisms in subsequent cultures, owing probably to the innumerable bacteria which were present

making it possible to obtain pure subcultures In a few instances this was accomplished by spreading the suspected pus, diluted with bouillon, over a large area of sterilized bread

A proliferation by budding, such as occurred in the body, is at first observed on mediums, but sooner or later the production of hyphae and myceliums characteristic of the mold growths occurs Budding is favored by moisture and thermostat temperature, the other methods of growth by

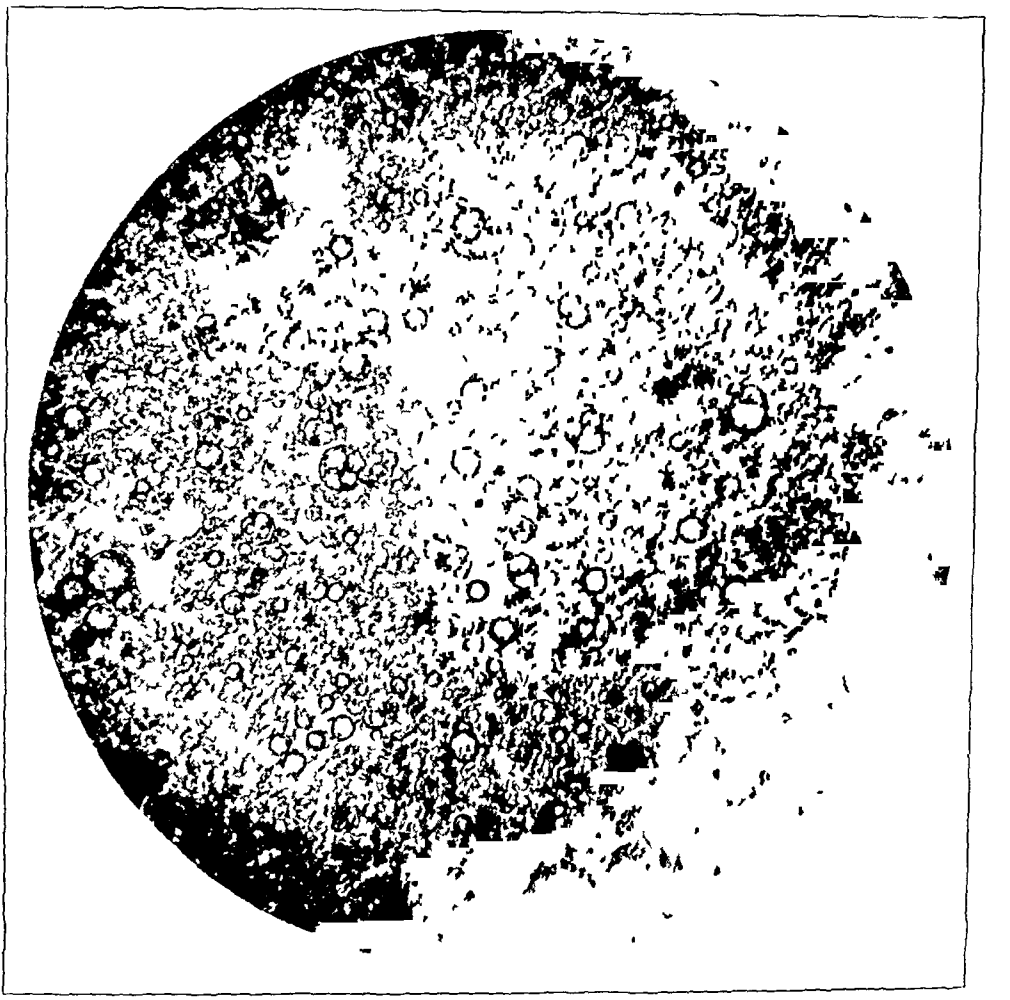


Fig 2—A preparation made from a blastomycetic culture which had become contaminated with *B subtilis* The innumerable small round forms suggest endosporulation

room temperature, dryness of the mediums and transplantation The time required for the appearance of the hyphal form varies somewhat In a few instances hyphae were noted in the first cultures made from the pus and kept at room temperature, in others the budding persisted for some time

The gross appearances of the cultures depend largely as might be expected on the method of multiplication, and many of the variations

reported are undoubtedly due to the degree of transition from budding to other forms of growth present at the time of observation. As mycelial growth becomes more marked the budding forms are less numerous and often difficult to demonstrate, but even after months of growth of the mycelial type on agar, a few budding forms may be found in the deeper parts of the cultures.

The pure budding form (blastomycetoid type) may occur primarily in cultures from pus or may appear later under certain conditions as a

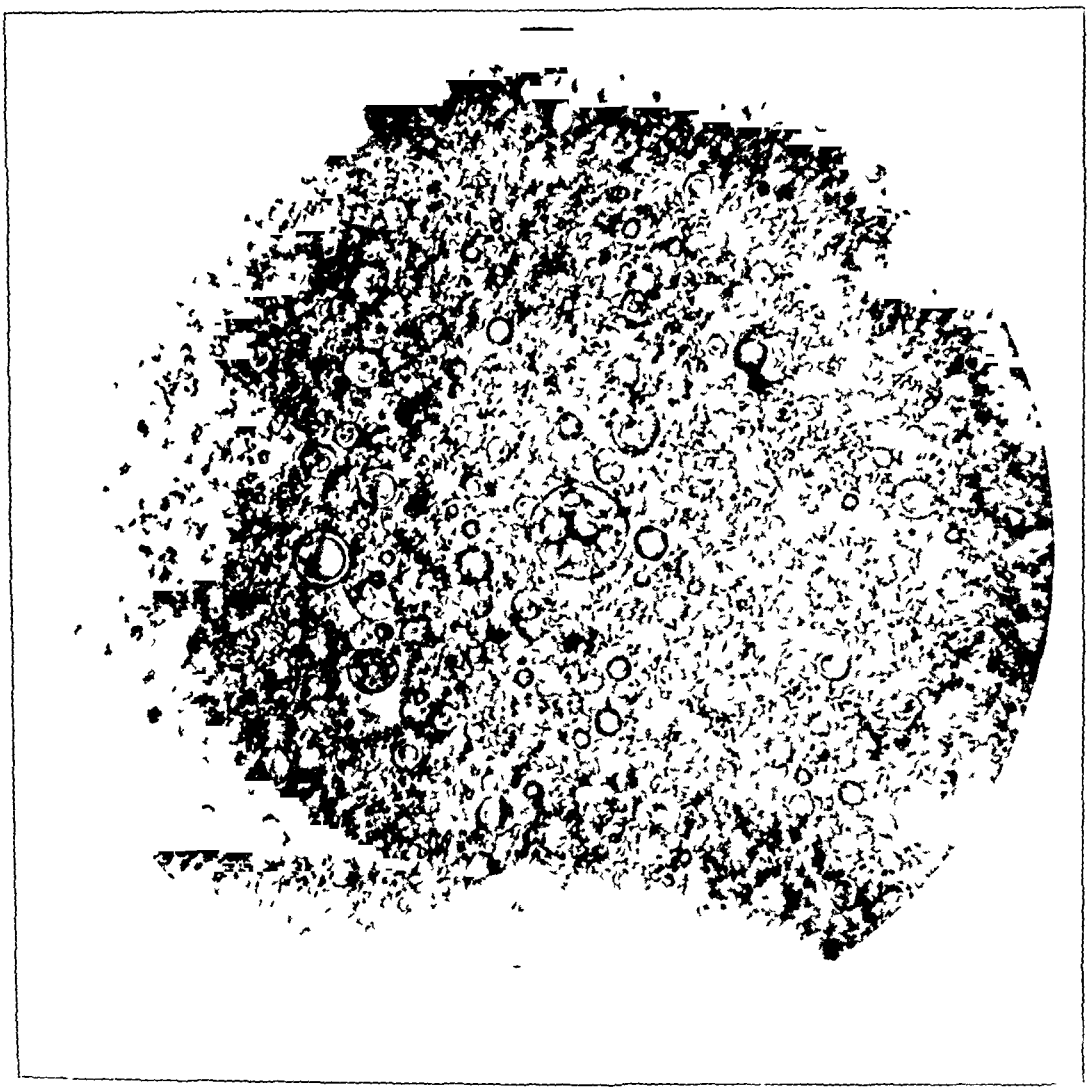


Fig. 3.—Endosporulation. Higher magnification. The innumerable small round forms are more plainly seen.

reversion from the mycelial form. The colonies on agar were at first small and round and presented a pasty appearance. They varied in color from white to light yellow and were easily detached and broken up. Later as hyphae began to form, they assumed a waxy or porcelain-like appearance, became tough and adherent and subsequently formed a surface membrane which in some tubes became nodular or rugose (Tubes *a* and *b*,

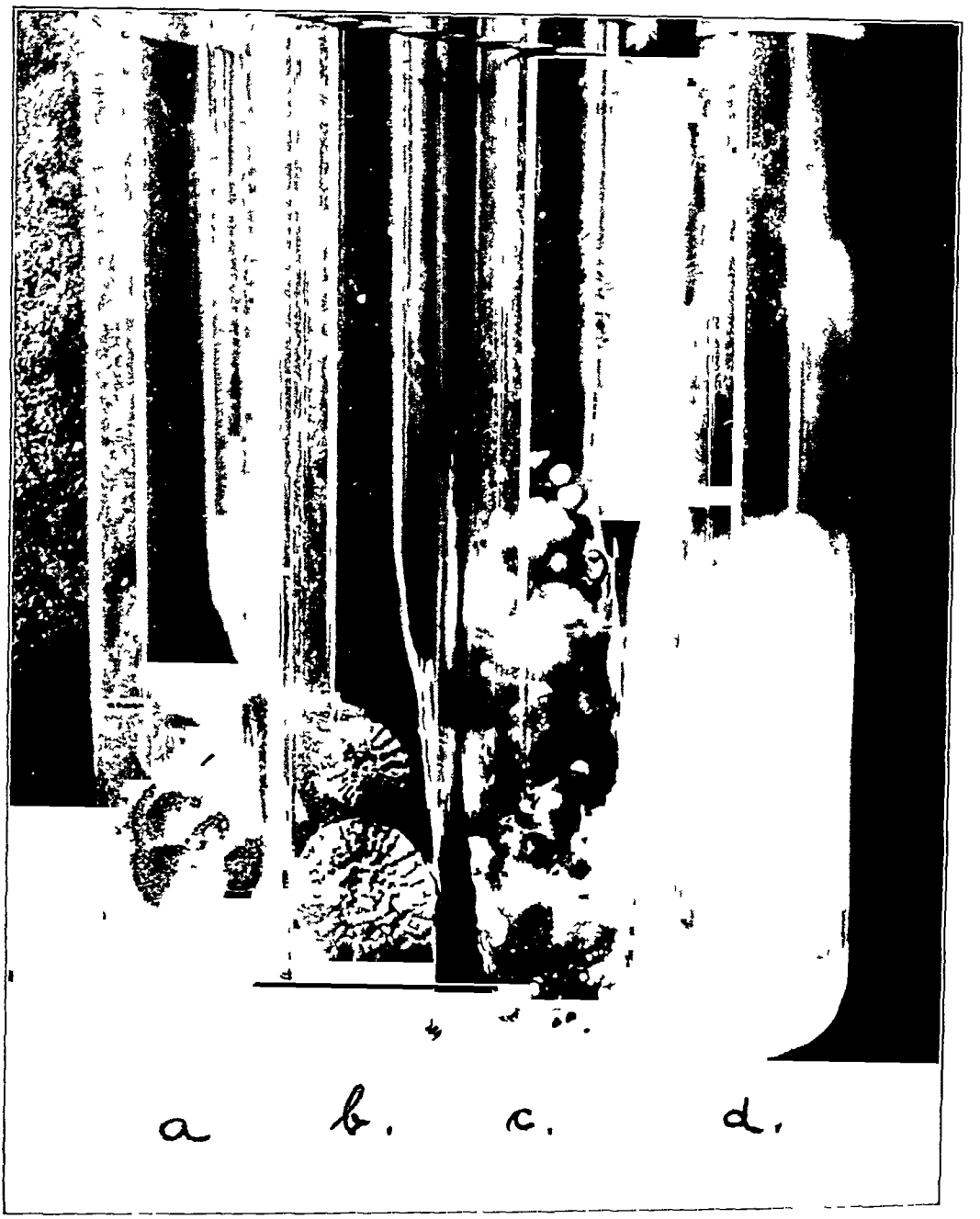


Fig 4.—Cultures of blastomycetes, Type 1 (*a, b*) nodular and rugose appearance of colonies on glucose agar, (*c*) mixed forms—beginning formation of aerial hyphae causing white fluffy appearances, (*d*) characteristic white fluffy growth of the mycelium with aerial hyphae



Fig 5—Cultures of blastomycetes Type 1, showing nodular, rugose and "bundle of worms" appearance

Fig 4), or assumed the "bundle of worms" appearance (Fig 5) Subsequent cultures usually presented the characteristic mycelial growth (Tubes C, D, Fig 4) It is of interest to note that such rugose or wrinkled surface membrane growths were more common during the fall and winter Indeed some of the strains which at room temperature during the summer grew abundant aerial hyphae, during the months of October and November grew these surface membranes which consisted principally of budding or short hyphal forms

Single colonies of the mycelial type appeared as fine branching hyphae, radiating from a faint yellowish-white center The hyphae grew into the mediums and also on and beneath the surface, increasing in number and extent until a membrane was produced Viewed directly, such colonies appear as a moist fine, meshed or felted network of branching hyphae, having a slightly raised center Viewed by transmitted light the extent and manner of the growth of the refractile mycelium can easily be seen (Fig 6) After longer growth the membrane may cover the surface and become very thick and adherent, the agar beneath often showing a brownish discoloration In some cultures the hyphae may not become aerial, and proliferation occurs by the segmentation of the mycelium into spores This corresponds to the oidium-like type of Rickett's classification Later in the growth, however, or in the subsequent subcultures, the hyphae in the center of the separate colonies or on the surface of the diffuse growths become disengaged or raised from the surface and may bear lateral and terminal fruit-buds This formation of aerial hyphae, giving the cultures the white, fluffy, mold-like appearance, occurred sooner or later in all the strains, and was for a time regarded as the final typical stage in the life of the organism (Tube D, Fig 4) The aerial hyphae, at first short and thinly scattered over the surface, often become luxuriant, growing up the sides of the glass and sometimes filling the test-tube In some cases aerial growth occurred in the first culture made from the pus This form corresponds to the aerial or hyphomycetoid type in Rickett's classification It will therefore be observed that the various forms described above, on which the classification of distinct morphological types was based, represent different stages in the life history of the organism A gradual transition from the budding form (blastomycetoid) to the submerged and surface mycelial (oidium-like) growth, the hyphae of which later became aerial (hyphomycetoid), can be seen in any one strain, if observed for a sufficient length of time These three forms, then, hereafter will be considered as variations of blastomycetes, Type 1, in contradistinction to Type 2, another form to be described, showing radical differences and representing, so far as has been observed, a possible final stage of growth or reversion type

Glucose-lactose agar gave much more luxuriant growths than the plain agar No differences could be observed in tubes of 0 6 per cent acid, neutral and 0 6 per cent alkaline reactions Growths on potato, gelatin and serum agar showed no characteristic differences, except that on the latter, which contains glucose, the growth was more luxuriant and wrinkled surface membranes occurred more often

Growths in bouillon inoculated with pus appear as fine sediment in the bottom of the tube and in shaking can be seen to be finely granular On examination, this consists of budding or short hyphal forms Later, a number of round discrete mycelial tufts closely resembling thistle-down appear In the test-tube these soon become confluent, forming a coherent mass, which can often be removed entire with the platinum wire A single colony grown in the flask increased in size until it filled that part containing the medium, and retained its circular form The older mycelium in the center of such large colonies undergoes a degeneration and becomes translucent, giving the growth a hollow appearance When the mycelium reaches the top of the medium, a tough surface membrane is formed, on which aerial hyphae develop Sometimes before the development of the latter the membrane sinks to the bottom, becomes brown and gelatinous and finally disintegrates, while another may form on the surface In subcultures, in which the transplanted material floats on the surface or remains adherent to the side of the glass, a surface membrane only may occur

The form already mentioned as Type 2 appearing in subcultures of Type 1 was first obtained in a subculture on agar of an old bouillon growth of an organism from Case 22 In a rather diffuse growth of Type 1 a number of white small waxy colonies were observed and for a few days regarded as a contamination When an attempt was made to remove them for examination, they were found to be very hard in consistency and quite adherent The structure was mycelial in character and pure cultures were then obtained The appearance of this mixed culture and also the first pure culture are shown in Figure 7 The character of this new type corresponded to the growth obtained from the blood in Case 19, and for this reason was considered to have some relation to Type 1 This type was afterward obtained from the cultures made from the pus in Cases 19 20, 21, 22, 23, 25 and 26

The time of appearance of the Type 2 is variable It occurred immediately in cultures from the blood in Case 19, in five weeks in cultures (Type 1) from the pus in Case 25, and fifteen months in cultures from the pus in Case 18 Its occurrence seems to be favored by prolonged growth on liquid mediums and a gradual transition has been observed

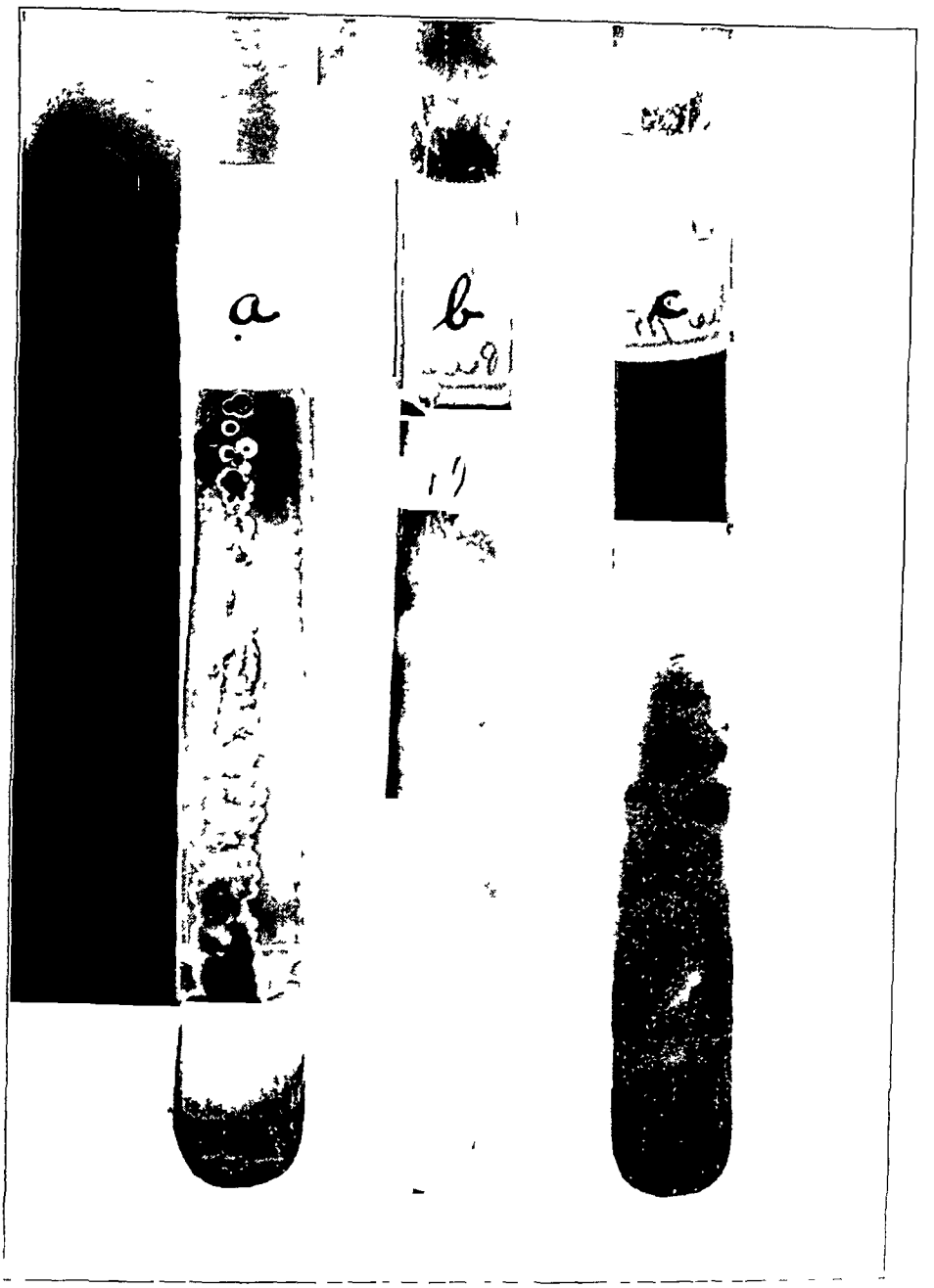


Fig 6.—Blastomycetic cultures viewed by transmitted light (*a*) Type 2, note the limited marginal growth of mycelium, (*b, c*) Type 1, submerged and surface mycelial forms whose manner of growth is more plainly shown

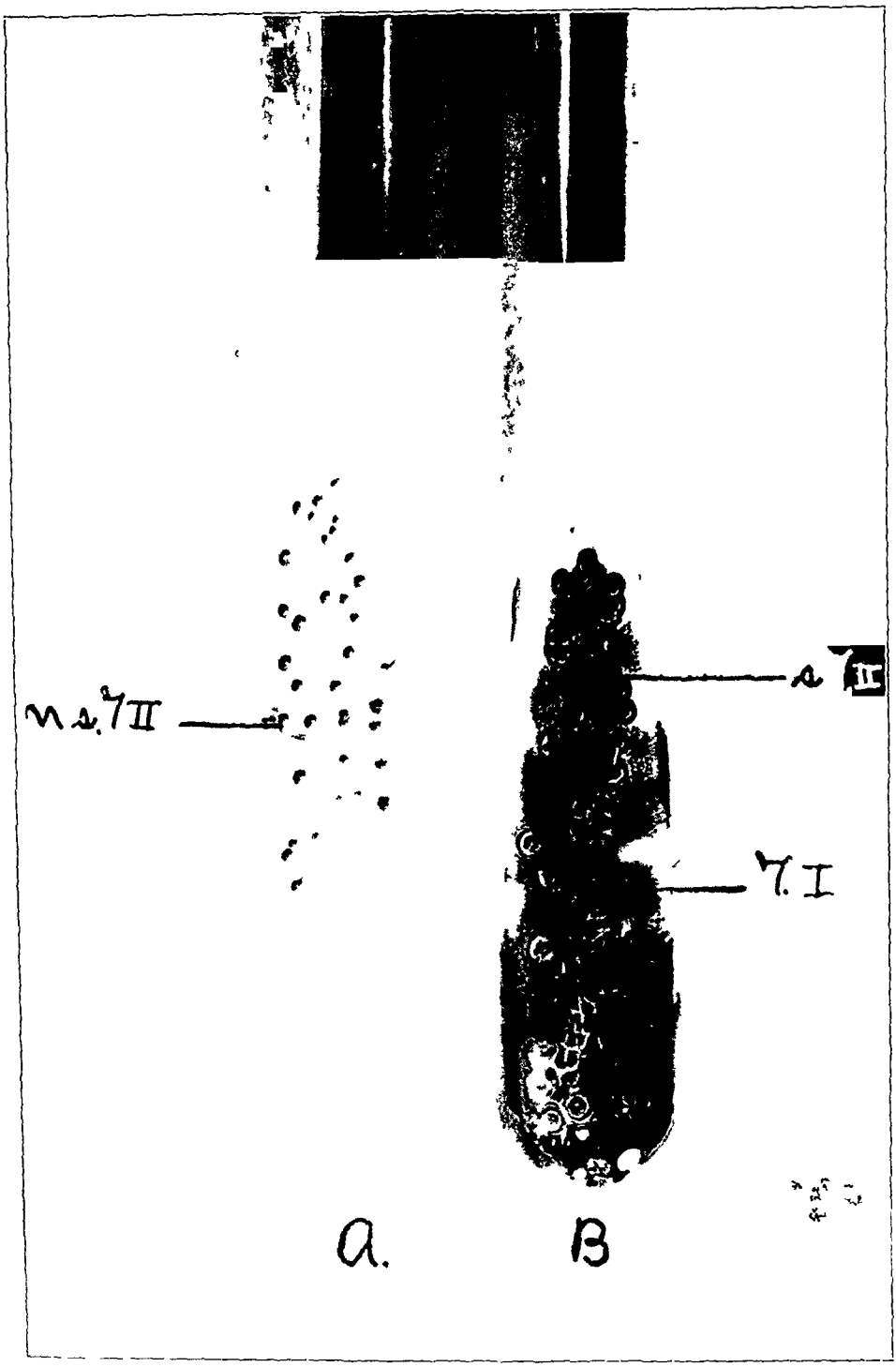


Fig 7 —Cultures of blastomycetes obtained from pus (*a*) non-sporulating form of Type 2 isolated form, (*b*) a culture of the usual Type 1 in which colonies of sporulating Type 2 (s T II) appeared

On agar the colonies of Type 2 appear as round, glistening, opaque, white or yellowish growths, resembling closely those of staphylococci. They differ, however, in that they are tough and adherent. As the colonies become older they sink into the medium and the margins are depressed beneath the surface. Occasionally a cleft or circular opening appears in the center, giving a burst-bubble appearance. The next change observed in the same tube or in a subculture is a marginal growth of hyphae, which by transmitted light appears as a halo about the colonies (Tube A, Fig. 6).

With plenty of moisture at incubator temperature, the discrete colonies may attain a diameter of a centimeter or more, the surface then becoming moist, slightly granular and wrinkled. When the colonies become confluent a membrane is produced which, owing to the rapid central growth, becomes wrinkled or corrugated similar to Type 1. The last, and so far as has been observed, the final stage, is the production of short hyphae bearing many small spores. The surface now presents a white powdered appearance (Fig. 8), although on glucose medium a gray has been noticed, and on several occasions a slight pink or red was observed. Glycerin medium, probably by preserving the moisture, seems to retard spore development. Reversion of Type 2 to Type 1 has not been observed. Type 2, then, so far as is known, represents the form finally assumed by the organisms after removal from the animal body, and is the form in which spore formation occurs to the greatest extent, resembling the natural molds in this respect. A minute portion of the spore form of Type 2 will produce innumerable colonies, whereas a considerable portion of the mycelium of Type 1 must be used to secure a growth. The possibility of obtaining the budding form by inoculation of animals with Type 2 is at present under investigation.

In cultures of Type 2 grown in bouillon the colonies are small, rarely attaining a diameter of more than a few millimeters. They usually remain discrete, and if they should become slightly coherent, separation readily occurs on shaking. In case any of the growths reach the surface a membrane also is formed, the surface of which assumes a white powdered appearance which is characteristic of the sporulating growths on agar. A distinct and characteristic musty odor is usually present.

Litmus Milk. The results of growths of either type on litmus milk were variable. In a few instances the milk became acid, but in the majority it was alkaline or unchanged. Coagulation occurred several times, but was not constant.

Fermentation Tubes. Both types of the organisms of the first five cases were tried with maltose, lactose, dextrose and inulin bouillon, but no gas formation took place. All but two of them were tried later with dextrose and lactose bouillon with the same result.

The organisms resist drying for prolonged periods, growths having been occasionally obtained from dry cultures over eight months old The presence of bacteria in the cultures seems, however, to inhibit the growth, and finally destroys the blastomycetes The organisms are also readily killed by heat, 100 C (212 F), 1 per cent, dilution of liquor formaldehyd and chloretone, 0 8 per cent, and phenol (carbolic acid), 5 per cent solutions Sunlight and lack of moisture also inhibit the growth

Morphology of Cultures The morphology of blastomycetes Type 1 in cultures has been so well described and illustrated by other writers[8] that nothing of importance can here be added, and the structure of Type 2 is so minute that a detailed description is difficult to give So far as has been observed, the mycelium corresponds in nearly all details to that of Type 1, except that it is very minute, the individual hyphae appearing as fine lines, with even the 1/12-inch lens The spores appear very similar to cocci Figure 9 shows fairly well the general appearance of the mycelium in contrast to that of Type 1 (Fig 10, same magnification)

Experiments with animals to demonstrate the pathogenicity of the culture we isolated were not so successful as some reported by other observers Dogs, rabbits, guinea-pigs, white and gray rats and white mice were used The material inoculated consisted of pus aspirated from abscesses, and both fresh and old agar and bouillon cultures The quantity varied from 1 to 7 c c, and this was injected subcutaneously, intraperitoneally, intravenously and intratracheally Death never was produced except when the cocci were present in the material inoculated A number of white mice died apparently from the trauma of the injection Subcutaneous inoculations usually produced abscesses, sometimes enormous in size, all of which healed spontaneously with or without rupture Demonstration of the organisms in these abscesses and their cultivation was possible only during the first few days following inoculation Injection into serous cavities or the veins produced a transient illness Animals killed at varying periods after intravenous inoculation showed no microscopic systemic lesions From agar-plates of the heart-blood of one rabbit killed two days after inoculation luxuriant colonies were obtained I should conclude, therefore, that the lower animals are markedly resistant to blastomycotic infection in spite of its power to produce suppuration It is possible that in the human being the resistance must be considerably diminished before infection takes place

The obviously serious nature of the disease in man and the increase in the number of cases taken into the hospital naturally occasioned anxiety on the part of the attendants in regard to the possibility of contagion from the patient's sputum or the profuse purulent discharges, and the question of isolation arose During the spring of 1908 a slight

8 Ricketts, H T Jour Med Research, 1901, vi, No 3

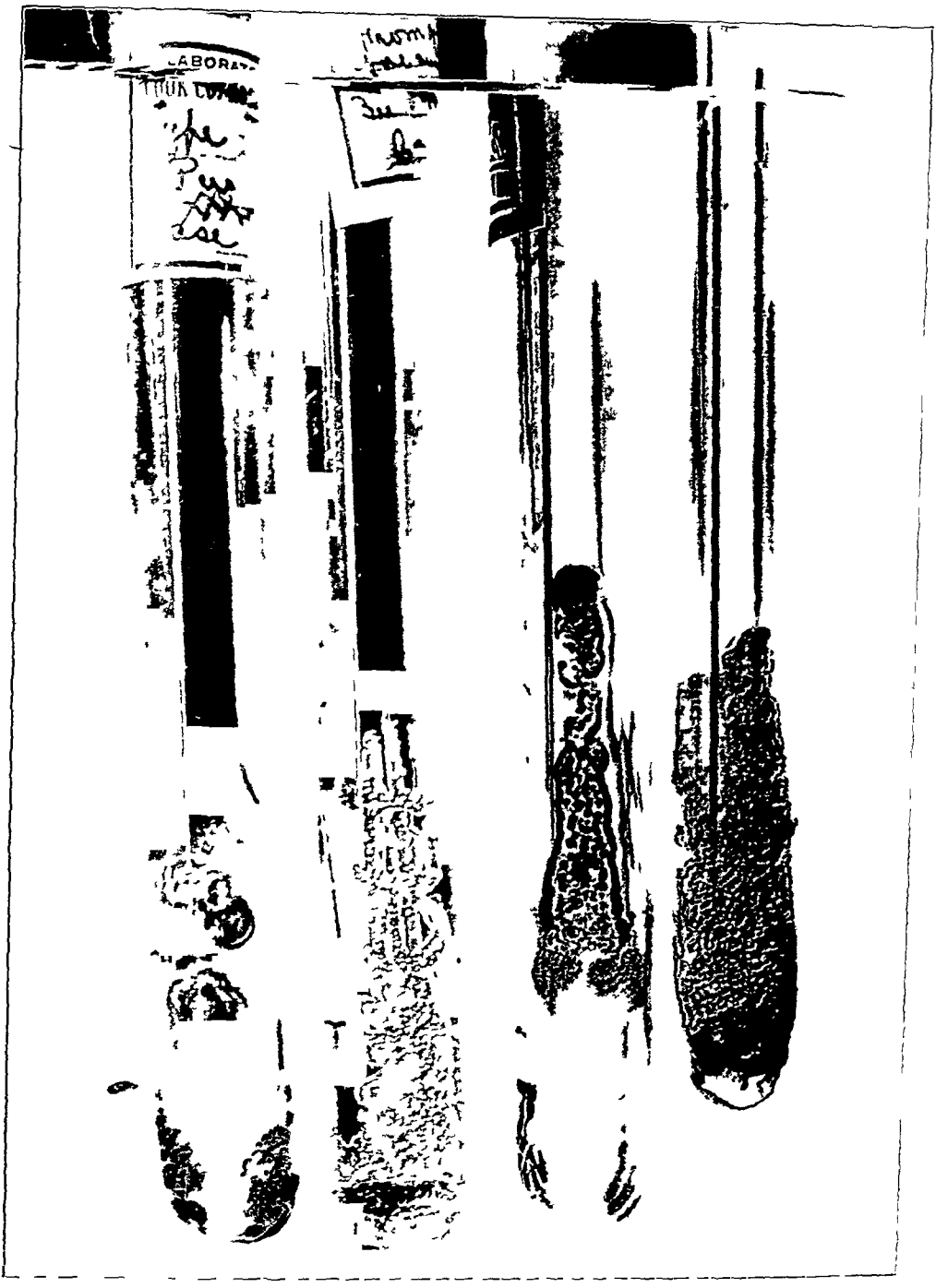


Fig 8.—Cultures of blastomycetes Type 2. Growths of Type 2 are at first smooth and waxy. Later, spores are formed giving the surface a white powdered appearance.

nasopharyngeal infection was present among the hospital attendants The throats of fifteen who had directly or indirectly been exposed to blastomycosis were examined No organisms were found in the pharyngeal or bronchial mucus, nor were any cultures obtained Strict isolation was not established, but reasonable precautions were observed To our knowledge no one has been infected, and it is improbable that infection with the organism as it exists in the pus or sputum occurs except by direct inoculation It may be advisable to mention at this point an incident which might have a bearing in the danger of handling the cultures

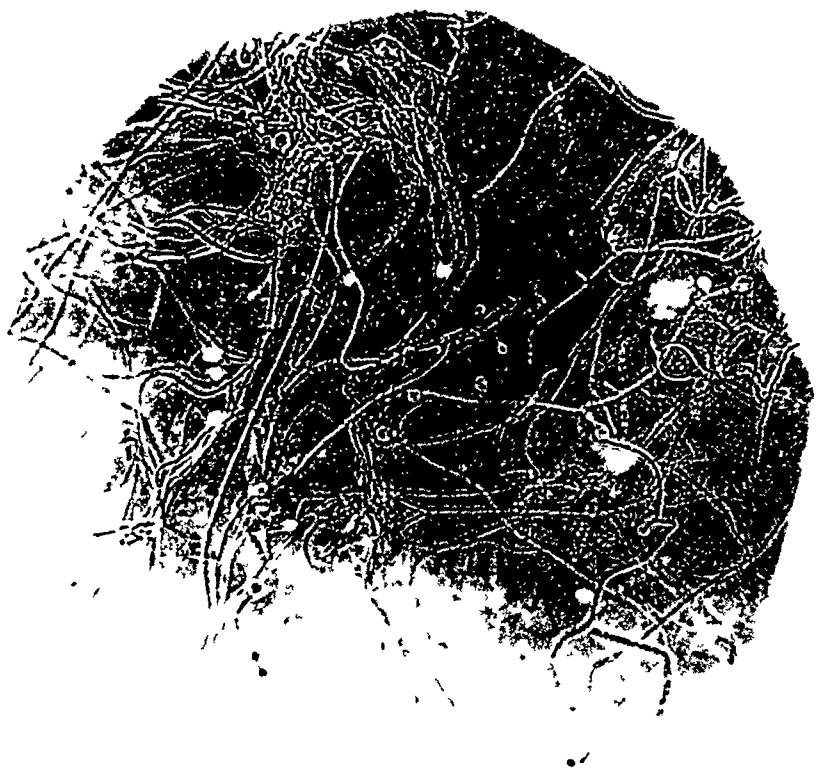


Fig 9—Photomicrograph of mycelium from a culture of blastomycetes Type 2 It presents the same general structure but is much smaller than Type 1

During the laboratory work a test-tube containing a bouillon culture of blastomycetes (Type 2—sporulating) from the blood of Case 19, was broken in the presence of another person and myself The odor was very irritating, and it is not improbable that a number of the spores were inhaled In spite of sprays and an antiseptic gargle, a pharyngitis and laryngitis developed in one of us The other experienced a chill about six hours later and a purulent bronchitis developed lasting for three days associated with considerable fever No organisms, however, were obtained in films or cultures made from the sputum

The resemblance of growths in cultures of these organisms to those of natural molds, and the ease with which (like the latter) they could be grown on moist bread (Fig 11), cotton and various forms of cloth and paper, led to an investigation of the home surroundings of several of the patients as well as the conditions prevailing in such places when the disease began The results are especially noteworthy, since in nearly all the reported instances in which the onset of the disease could be



Fig 10.—Photomicrograph of culture of blastomycetes Type 1 It shows plainly the budding and hyphal forms

determined, it was learned that the first symptoms developed during the months when in this region great dampness and mold growth prevails

One patient (Case 19) lived in a basement on Huron Street At the time of investigation it was uninhabited and entrance could not be effected Through a broken window, however the rooms were seen to be very dark The floor was laid directly on the earth and a number of the boards were completely decayed From the neighbors it was learned

that the family had very unhygienic habits, and that owing to lack of room the patient had often slept outside under the sidewalk Another (Case 20) lived in South Chicago, also in a basement with a low ceiling and painted walls The wooden floor was several feet below the level of



Fig 11—A loaf of bread three weeks after inoculation with blastomycetes Type I The mold has spread over the entire loaf

the street, and the surrounding land was marshy and covered with pools of water When examined (November) the rooms had been renovated and were in a fairly sanitary condition The wife however, stated that after the snow came and the ground was frozen the floors were always

damp, and that this was increased by the condensed steam trickling down the walls, also that the bed-clothes became so moldy that each spring they were forced to burn the mattresses A third patient (Case 21) lived on the second floor of a three-story building The basement, in which the closets of the tenants were situated, was dark, requiring the use of candles in the daytime The floor of this basement was partially flooded with water, which was escaping from the sewer-pipes The planks covering the floor were in various stages of decomposition, and presented much mold growth

The fourth patient (Case 22) for six months prior to the onset of his illness had lived in a cabin-boat while engaged in dredging A fifth



Fig 12.—A flash light photograph of a basement in which one of the patients slept during the winter he was infected His bed was made of old carpets on a pile of sand near this board wall, which was banked with earth on the outside For several inches above the point where the boards (*bo*) entered the earth (*ea*), a brown fungous growth (*mo*) was present Above this, for some distance on the board the fungus was dry and the surface was covered with spores, giving it a white powdered appearance (*sp*) The growth was most luxuriant during fall, winter and spring months

patient (Case 23), during the winter he was infected, slept in a basement located in the "Ghetto" on the West Side of Chicago It was low, dark and poorly ventilated The wooden walls were backed with earth, quite moist in places and covered with a brown fungus growth Where the wood was dry a white powder-like deposit was present (Fig 12) The

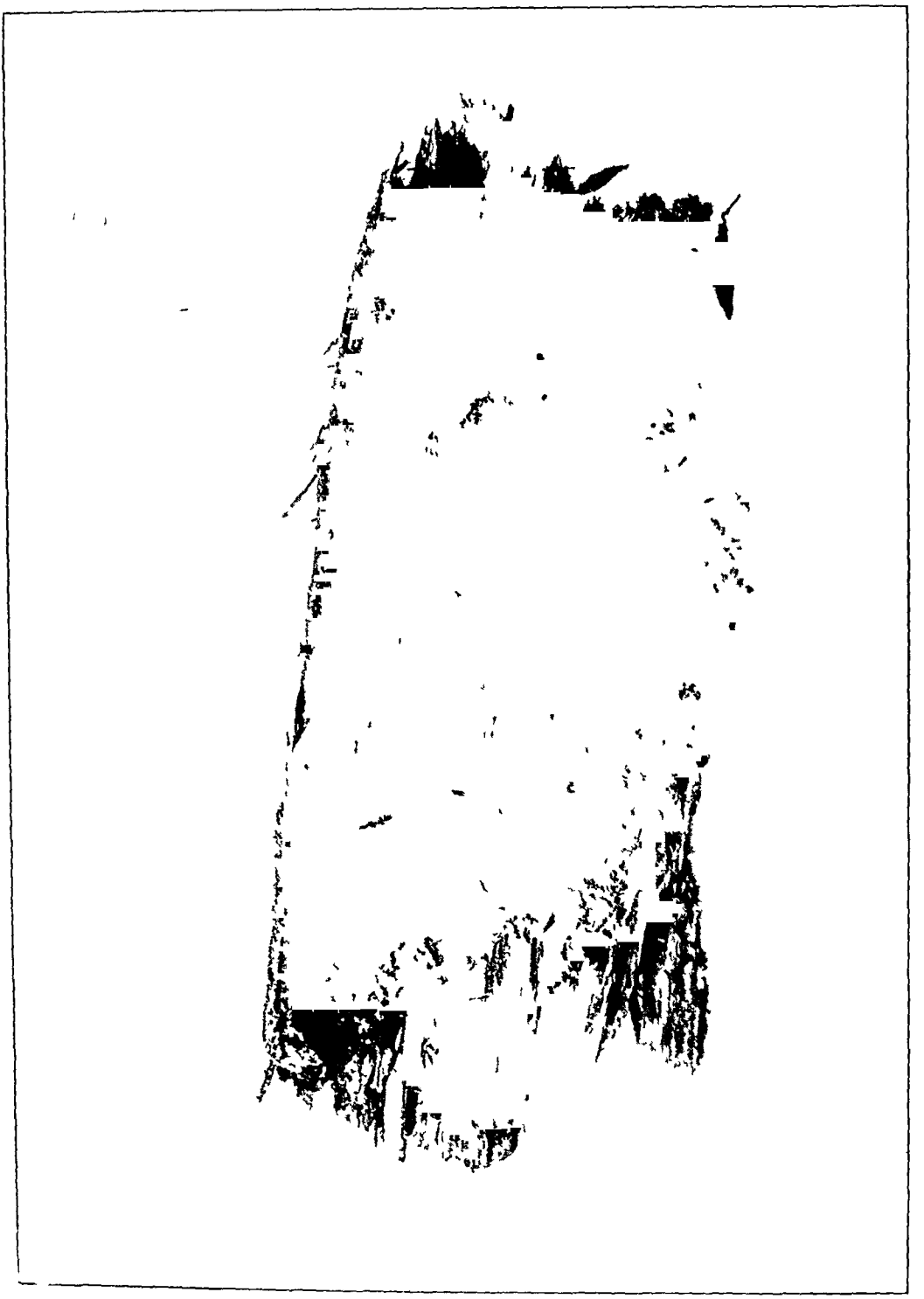


Fig 13—Fungus growth on a piece of wood removed from the basement illustrated in Figure 12

patient slept on a pile of dirt near this wall, and his coverings consisted of several carpets which were also moldy Several pieces of the decayed boards were removed and taken to the laboratory The general appearance of the fungus growth was not unlike that of brown plush (Fig 13) On drying the surface became covered with a white, powder-like deposit (Fig 14) The same changes took place on the walls of the basement during dry weather This powder when inoculated on agar gave pure cultures of a mold which differs in only a few respects from the blastomycetes, Type 2, to be described presently Inoculations from the bouillon in which this fungus has been triturated also gave a large number of colonies of this type, and a few of another type A low-power view of a section of the wood and attached fungus is shown in Figure 15 The general mycelial structure in which compartments of spores exist can be made out as the magnification is increased, and two varieties of circular bodies can be recognized[9] (Fig 16)

A sixth patient lived near the lake in South Chicago, on the second floor of a two-story frame house built from 3 to 5 feet below the street-level and covered with water during the winter and spring One small cooking-stove supplied the heat in winter for the rooms occupied by sixteen men When examined in August the room, which had painted ceilings, was dry and well aired During the drought of July and August, 1908, the dirt floor of this basement was still wet, and white mold spores were found on the timbers[10]

The residence of a seventh patient (Case 25) could not be located It was learned, however that he had lived in the Croatian colony in South Chicago for three months The dwellings there are old frame houses built on ground so low that the first floor is 3 feet below the street level

9 The first is a clear, round, translucent body, and is the variety which is most abundant They are produced by segmentation of the hyphae, and probably constitute the spore form The second variety is larger and usually dark brown A few of these can be seen in the surface mycelium, but they are most abundant in the substance of the wood Some appear to be formed in the hyphae, and in other places the hyphae seem to originate from them Except in color they resemble closely the blastomycetes found in pus In Figure 17 the double contoured round and budding forms can be seen distinctly A teased specimen of the surface mycelium shows branching and segmentation similar to that observed in cultures of blastomycetes (Fig 18) The first mentioned cultures differ only from blastomycetes in that the growth is more rapid and luxuriant and that a red color appears in the growths on glycerin agar, although a slight pink has been observed in the growths of Type 2 obtained from the blood cultures The second variety obtained from the wood resembles Type 1 in appearance but it bears abundant elongated or cigar shaped spores In later cultures these spores become round Photographs of these cultures are seen in Figure 19 Note resemblance to cultures of blastomycetes (Figs 4 and 8)

10 Some of the material was removed to the laboratory and nearly pure cultures of a mold resembling those already mentioned were obtained

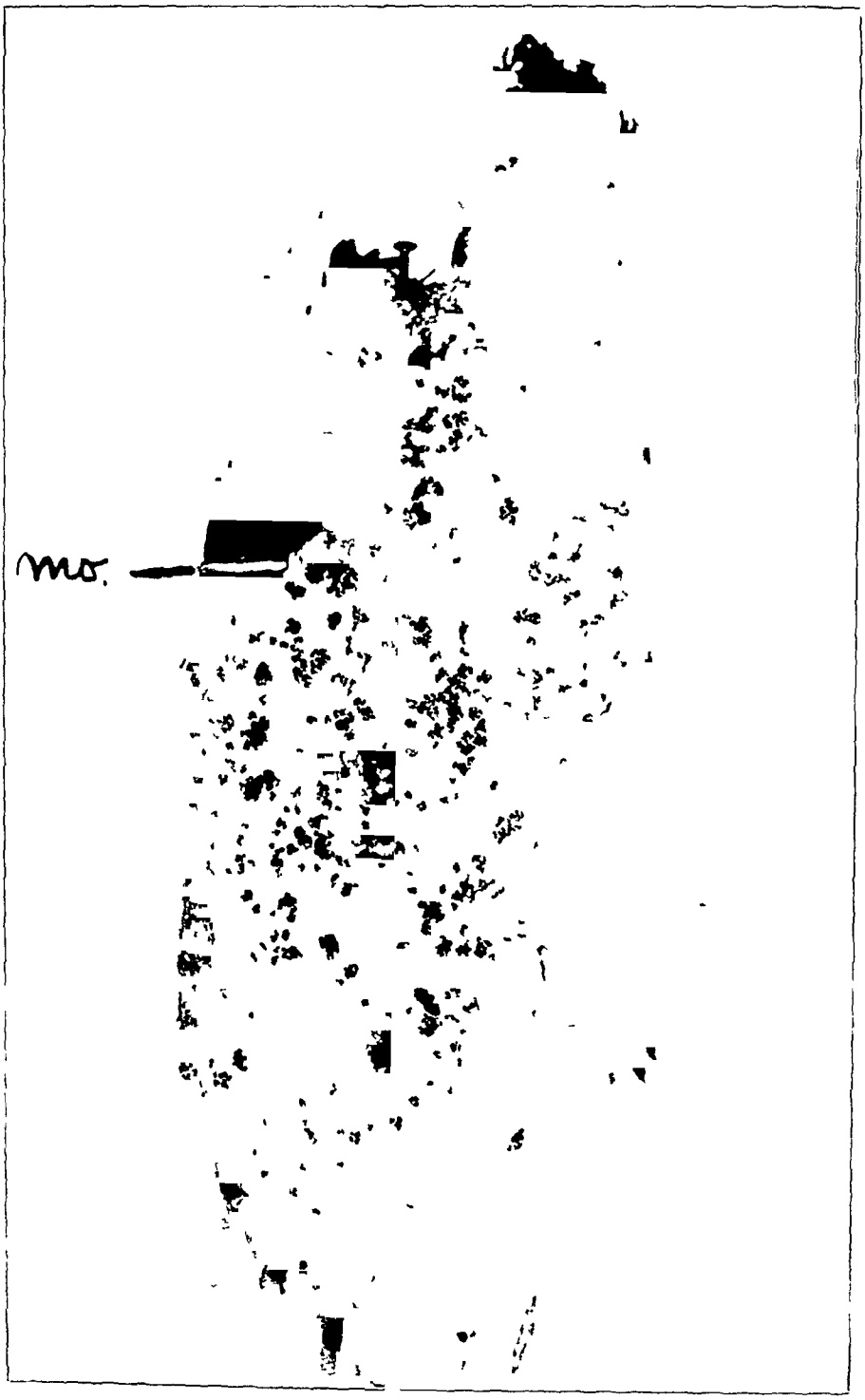


Fig 14 —Another piece of wood from the same basement The photograph was taken several weeks after it was removed to the laboratory and shows the numerous areas of white fluffy mold (*mo*) which appeared

An eighth patient (Case 26) lived on the North Side, on the second floor of a two-story frame building, the first floor was used as the wash-room of a steam laundry in which the patient worked The floors were old and necessarily damp No molds were discovered in this room, but in an adjacent closet a profuse mold growth was found on the floor Immediately under the window of the bedroom was a small shed used as a storeroom, and often frequented by the patient The floor at the time of investigation (July) was covered with litter which was damp and moldy The occupants stated that during the fall, winter and spring,



Fig 15.—A low-power field of a section of the fungus illustrated in Figures 23 and 24 (*wo*) wood fibers, (*hy*) mycelium made up of hyphae enclosing (*sc*) spore compartments, in the latter are a large number of (*sp*) spores

water was always present under the floor and could be forced up between the cracks of the boards by the weight of the body Two barrels of decaying manure stood just outside the door [11] No mold could be found in the living-rooms upstairs It was learned, however, that during the winter the walls were always wet from the condensed steam of the laundry

11 It is noteworthy to mention that one of Dr Ormsby's patients at the time of infection slept in a room, the windows of which were located just above a manure pile

below The bedroom was so small that there was room only for the bed The only window was the one mentioned as located just above the shed and manure barrels Specimens of moldy wood and leather from the shed and closet were taken to the laboratory and cultures obtained which resembled those already described

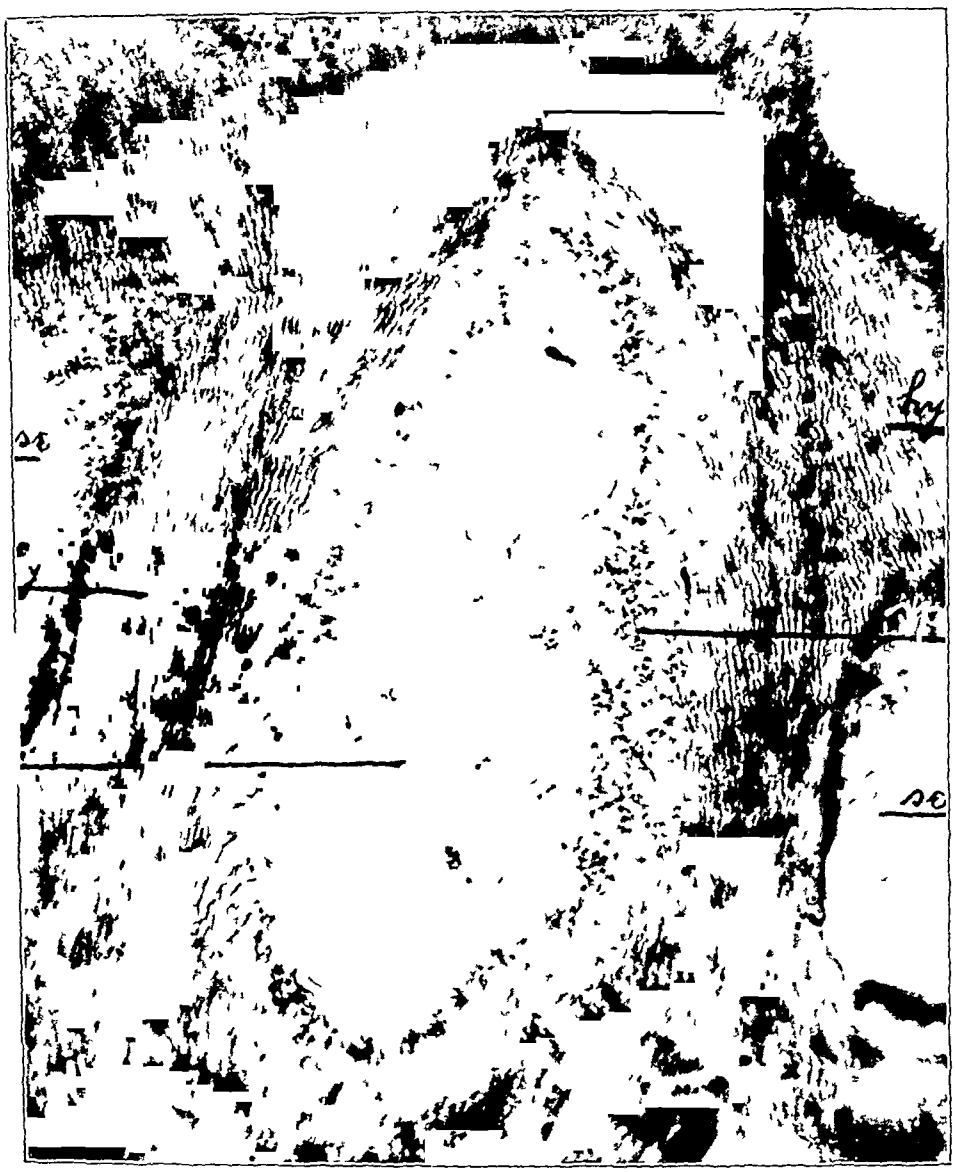


Fig 16 —A higher magnification of the wood mold showing more plainly the (*hy*) branching segmented hyphae, (*sc*) spore compartments and (*sp*) spores

The patient with cutaneous blastomycosis, mentioned subsequently, lived in a house the walls of which were constantly damp during wet weather The roof leaked to such an extent that it was necessary to provide vessels to catch the water He did not remember having seen any mold in the house, but states that musty or moldy odor was always present

In view of the uncertainty expressed in botanical literature as to the closely related organisms, and because no one has undertaken to determine the final place the blastomycetes shall occupy in the systemic mycology, it is hardly pertinent to more than suggest that like other molds these organisms may possess a native habitat in such insanitary dwellings as those from which these patients came It would also be hazardous to do more than suggest an identity between the molds grown from decaying boards and walls in these dwellings and the blastomycetes, although in many respects points of similarity were demonstrated, such as morphology, cultural reaction pathogenicity and vaccine reaction [12]

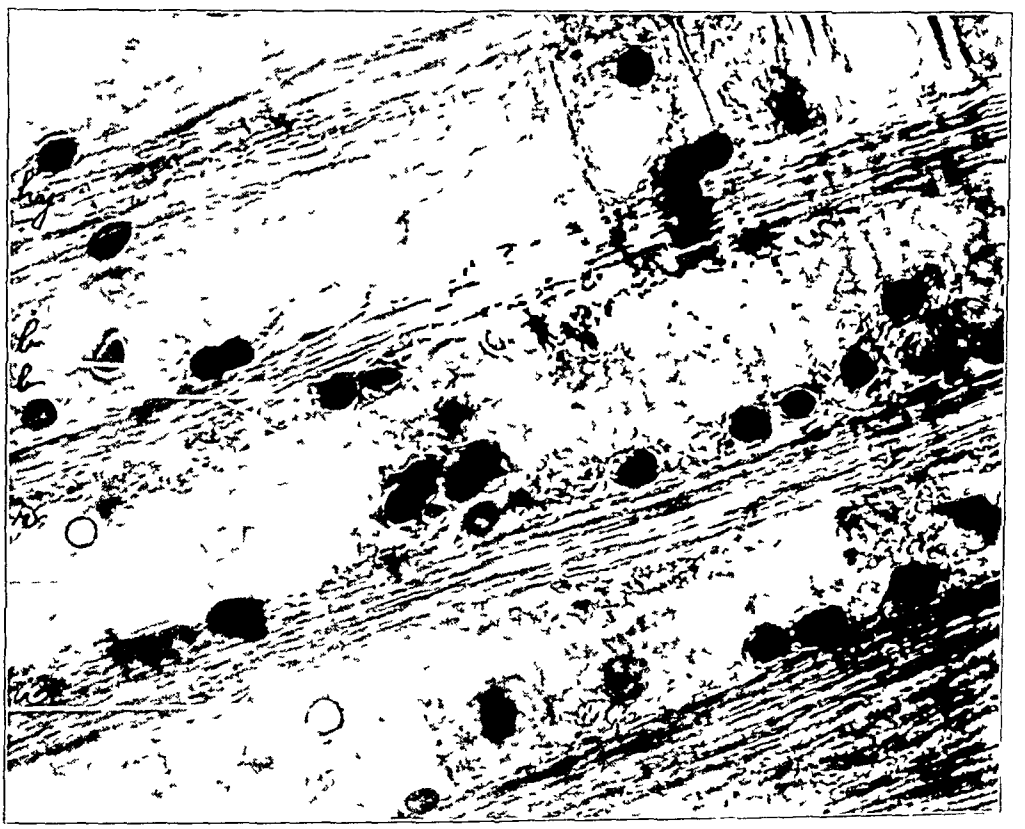


Fig 17 —Photomicrograph of the wood just underneath the fungus, showing round (*r*) and budding (*b*) forms of organisms with refractile outlines Segmenting hyphae (*hy*) can be seen running parallel to the wood fibers

The suggestion of infection from these natural sources by inhalation is perhaps justified to a certain extent in that it is generally held at present from both the clinical and pathological evidence that the respiratory tract is the atrium of infection in systemic blastomycosis In at least fourteen of the cases reported the first symptoms were referable to the lungs, and of eleven necropsies in these cases, nine presented old

12 See Case 6, vaccine therapy, also in a subsequent discussion in this article

bronchopneumonic lesions It is also possible that the subcutaneous abscesses or cutaneous lesions marking the onset in other patients were due to unrecognized pulmonary lesions with metastases in the skin and elsewhere In man no very good evidence of infection of the intestinal tract has been obtained In four cases of this series the Peyer's patches were reticulated and pigmented, and in two cases the mesenteric lymph-nodes were enlarged In another case (Case 7) peculiar nodes were found in the colon and regarded by the authors as blastomycotic, organisms,

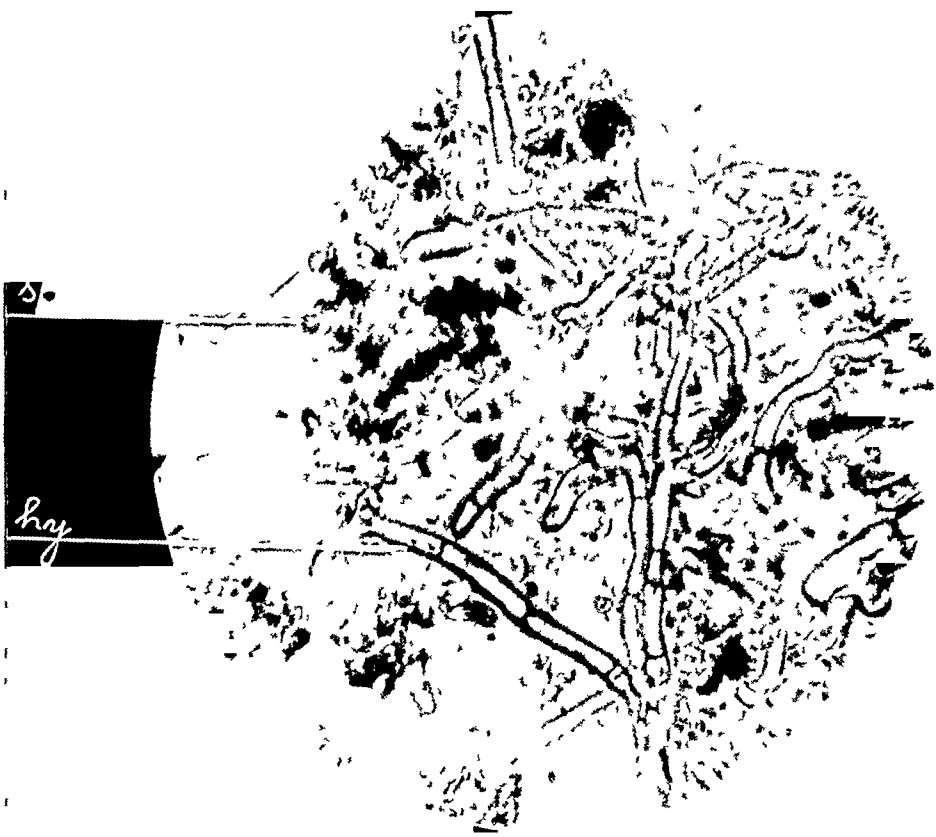


Fig 18—A teased bit of mycelium from the wood mold showing the segmentation of the branching hyphae (*hy*) and the round spores (*sp*)

however, have not been demonstrated in either the bowel or mesenteric lymph-nodes

As for infection of the skin, such a large number of cases of cutaneous blastomycosis have been reported that this condition is no longer regarded as uncommon In some of these it seems quite evident that the infection followed some form of traumatism In only three instances however has it been clearly established that systemic infection followed, and in these the cutaneous lesions existed for from seven to twelve years

respectively, before symptoms of systemic blastomycosis were manifested In once case (Case 12) the infection occurred several months after cutting the foot on a clam-shell That direct inoculation does occur was established in two of our cases in which new superficial lesions developed in the abrasions resulting from scratching Blastomycetes were found in the detritus under the finger-nails of these patients and undoubtedly had origin from preexisting ulcers which the patients persisted in scratching

Pathology The skin and lungs have already been mentioned as constituting the principal atria of infection The spread of the organisms from these points and the resulting pathological conditions depend very much on the resistance of the individual and the location of the primary lesion

Dissemination of organisms from cutaneous lesions does not seem to have occurred very often These lesions have considerable tendency to spread by contiguity of tissue, but invasion of adjacent lymph-nodes is not common, and in only a few instances have the organisms entered the blood-stream, producing metastatic lesions in the lung and systemic infection In two cases this process seems to have followed directly the curettement of the cutaneous lesions

Primary infection of the lungs, as pointed out before, appears to occur first in the bronchi, to be followed by the development of areas of bronchopneumonia in the neighborhood Further lymphatic spread is possibly responsible for the pleuritis, pericarditis and mediastinal lymphadenitis which have been noted From these lesions in the lung, organisms may enter the blood and become distributed throughout the system with the production of multiple metastatic foci, which in turn may give rise to further dissemination by the blood route and reinfection of the lung The repetition of these processes may thus constitute a vicious circle and afford ample explanation for the numerous metastatic lesions characteristic of this disease The skin, muscles, subcutaneous tissues and the bones of nearly every part of the body have been involved, and all of the important viscera have presented more or less evidence of infection

The variation in the reaction of the tissues to the presence of the organisms noted in a single case are most remarkable Blastomycetes in considerable numbers were not uncommonly found associated with no histological change whatever In other instances slight necrosis was the only alteration noted

The majority of the infected areas, however, presented round-cell infiltration, either alone or associated with giant-cells, polymorphonuclear leukocytes, or both In some tissues, especially in the viscera, in which the lesions were small, definite nodules were found and closely

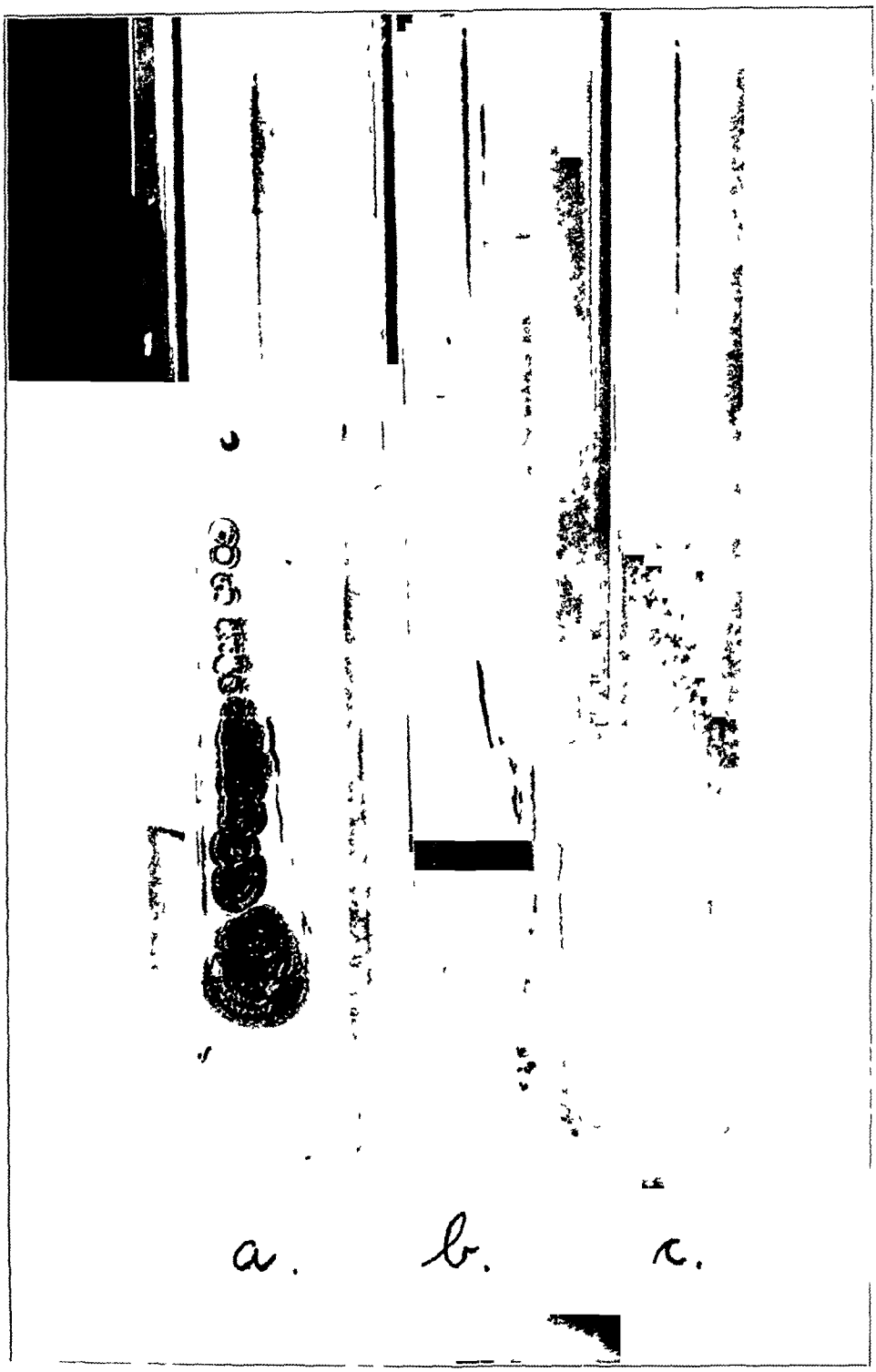


Fig 19 —Cultures made from the wood mold gave a large number of colonies (*a b*) closely resembling those of blastomycetes Type 2 obtained from blood and pus A few colonies resembling blastomycetes Type 1 were also obtained and shown in tube (*c*), (*a*) and (*b*) are the same except that the former is the older culture and sporulation has occurred, giving it a white powdered appearance

resembled those found in tuberculosis except that central necrosis was less marked Other nodules contained many polymorphonuclear cells constituting small abscesses These were found either discrete or confluent the latter being separated by blood-vessels and bands of compressed tissues Some were very small, occupying an area equal in diameter to one-half of a high-power field The larger abscesses were often surrounded by a distinct wall of granulation-tissue Giant-cells usually were present, often in considerable numbers Their absence in a few instances was especially noted They were usually of the Langerhans type, but were rarely as typical as those found in tuberculosis In some cases heal-



Fig 20 —A section showing blastomycotic abscesses in the skin and subcutaneous tissue (*sk*) skin, the epithelial layer of which has been involved secondary to (*ab*) the subcutaneous abscesses, (*ab'*) an abscess about to rupture, typical cutaneous lesions often develop at these sites

ing of the infected areas occurs and is often associated with marked fibrosis, especially in the skin, lungs and pleura

The changes most characteristic of blastomycosis are the cutaneous ulcerations, the deep and superficial abscesses, and the often tubercle-like nodules in the viscera Some of these lesions deserve special mention

The infections in the skin may appear as pustules, subcutaneous abscesses or local ulcerations (Figs 20 and 21) The pustules are usually multiple and may occur successively or in crops The ulcers

may occur primarily as such or develop in the site of the ruptured abscesses In cases of the latter, the ulcer produced is usually surrounded by an areola of inflammation, the margins are slightly raised and irregular, and the base is soft and covered with granulation-tissue, which may become fungoid and approach the surface, later assuming a papillomatous appearance, which is more or less characteristic of the lesions of chronic blastomycosis of the skin (Fig 22)

The abscesses may be superficial or deep and have origin in the skin, subcutaneous tissues, lymph-nodes, muscles, bones, nervous tissues and viscera Their size is most variable, ranging from very minute areas to



Fig 21 —Photomicrograph of a section of subcutaneous abscess (*a*) stratum corneum (*b*) papillary layer which is being invaded by (*c*) the abscesses containing large number of blastomycetes, infection here occurred from the blood, (*d*) subcutaneous tissue

enormous cavities containing a quart or more of pus Those having origin in the deeper tissues are more or less hard at first, but fluctuation develops sooner or later Some are associated with pain and the signs of inflammation, whereas others are discovered by the patient accidentally Spontaneous healing of even large abscesses was noted, but the majority either ruptured or required incision and drainage to relieve the tension pain Healing then took place or a discharging fistula persisted, the opening of which sometimes became surrounded by the char-

acteristic papillomatous ulceration The pus usually was thick and tenacious, making aspiration difficult, and varied in color from a light yellow to a dark red or brown, according to the amount of blood present

Blastomycetes can usually be demonstrated in the pus by mounting it direct or mixing with a 20 per cent solution of sodium hydroxid (Fig 1) In a few instances they could not be demonstrated either in smears, tissue sections or by cultures Perhaps the most reliable method in such cases is the staining of a thin smear on a slide with hematoxylin and eosin, or with polychrome-methylene-blue The degenerated forms can then be more easily recognized

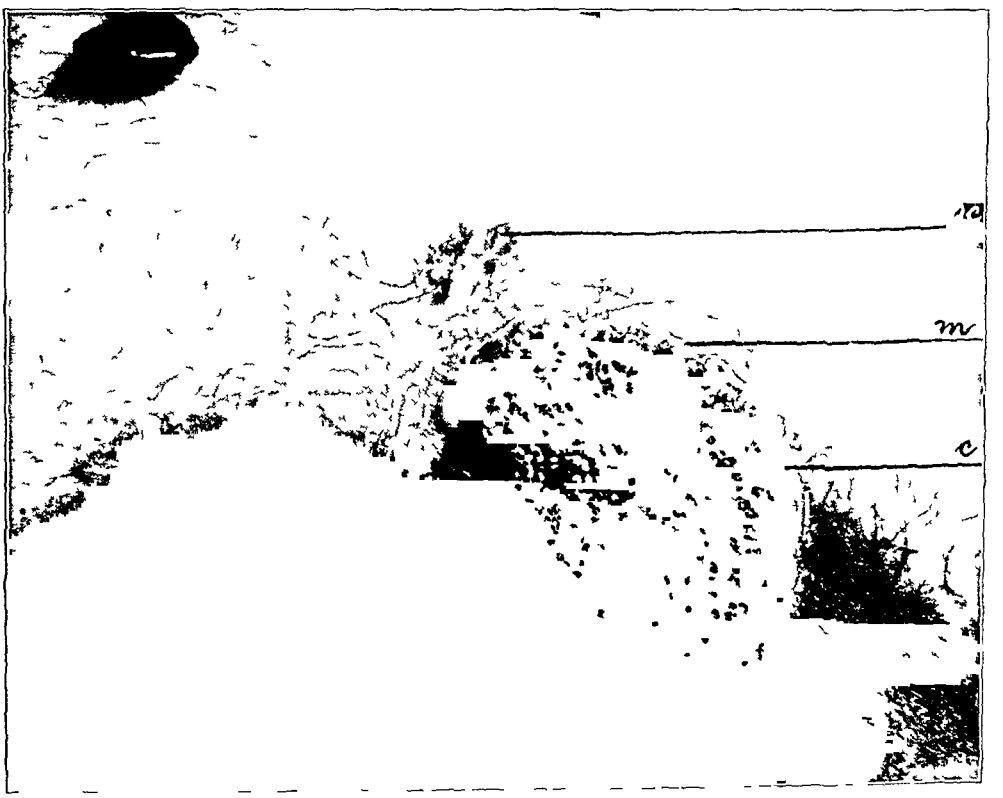


Fig 22.—A typical lesion of cutaneous blastomycosis (*c*) papillomatous or verrucose surface, (*m*) raised indurated border containing miliary abscesses, (*sc*) scar of old lesion (By courtesy of Dr O S Ormsby)

The affections of the bone consisted of osteomyelitis, periostitis and arthritis The osteomyelitis which is suppurative in its nature may be diffuse[13] or localized In either case the fungi are very numerous, invading the narrow compartments and causing necrosis of the marrow cells with the formation of a purulent exudate[14] The bone partitions then disappear, the areas become confluent and an abscess is thus pro-

13 Figure 2 Case 19 article by Krost, Moes and Stober, this issue
14 Figure 4, article by Krost, Moes and Stober, this issue

duced The process may involve the bone to such an extent that spontaneous fracture happens Ruptures of these abscesses often occurred, causing extension to adjacent tissues Such lesions in the ribs,[13] clavicle, sternum,[15] the bones of the skull, and the small bones of the hands and feet were important features in a number of cases Formation of sequestra was observed in two instances

In the long bones the infection has a tendency to lodge in the epiphyses,[16] and in some instances the shape of the infected areas strongly resembled those produced by infarcts[17] Extension by rupture of the epiphyseal abscesses was responsible for the majority of the joint lesions[18]

In those necropsies in which opportunity was afforded to study the changes in and about the joints, communication with abscesses in the adjacent bone were always found In the cases in which suppuration of the joint was marked, more or less destruction of the synovial membranes, the articular ligaments and periarticular tissues was produced, often to the extent that subluxation occurred The articular cartilages were sometimes so completely destroyed that the rough eroded bone was exposed[19] Diffuse blastomycotic osteomyelitis of such an exposed head of the tibia was found in Case 21[20] A healing periostitis was also present in this case Special mention should be made of the spinal lesions because of the close resemblance of the symptoms produced to those of tuberculosis or Pott's disease Spondylitis was present in Cases 7, 8, 18, 19, 23 and 28 The bodies of the vertebrae were affected in all except Case 23, in which the disorganization seemed to have occurred in the laminae and the posterior ligaments More or less complete paralysis occurred in two cases

A careful study of the appearances of the bone lesions in numerous roentgenograms was made by Dr H E Potter, roentgenographer to the Cook County Hospital, and the following are his observations

In general the presence of blastomycotic infection of bone is easy of demonstration on account of the intense localized nature of the destructive process In the first stages of the invasion no changes are visible, but as soon as destruction and absorption have advanced to a moderate degree the lesions are easily demonstrable On the whole it has been our experience that the radiographic findings are more marked than in other types of infection presenting a similar clinical picture Moderate pain, tenderness and swelling around a joint may be accompanied by a marked degree of bone involvement

15 Figure 1, article by Krost Moes and Stober, this issue
16 Figures 4 and 5 article by Lewison and Jackson, this issue
17 Figure 1 article by Myers and Stober this issue
18 Figure 3 article by Myers and Stober this issue
19 Figure 2 article by Lewison and Jackson this issue
20 Figure 4 article by Lewison and Jackson, this issue

I have been fortunate in being able to obtain numerous skiagraphs at various stages of the disease from cases which were being studied exhaustively from a clinical and pathologic standpoint The lesions which were skiagraphed and studied occurred in the spine, ileum, ribs, femur, tibia, fibula, scapula, humerus, radius, ulna, and in the wrists, hands, ankles and the feet The following conclusions have been drawn from a serial study of the plates alone

Skiagraphs of blastomycotic bone lesions show rarefactions and osseous proliferations not dissimilar in some respects to those seen in other types of bone infection A comparative study of more than a score of these lesions in their different stages and degrees of activity has shown, however, that certain appearances are quite constant in blastomycosis and but seldom seen in other affections

The lesions are most numerous and distinct in the long bones Here they are first seen in the spongy portion near the epiphysis (Fig 23) or, if the extremity bears no epiphysis, they lie in close relation to the articular surface (Fig 24) A focus may remain single or others may appear in the vicinity, coalescing as they increase in size Coincident with rarefaction a bony periostitis develops over the extremity of the diaphysis and may completely envelope it



Fig 23.—Lower end of radius and ulna *R*, an early focus in ulna beneath epiphysis (*E*) R' R'' and R''' are early foci in radius *P*, periosteal ossification

The newly formed bone may in turn undergo a progressive dissolution as the inner necrosis advances although an instance has been observed (Case 24) in which an arrested rarefaction was followed by a complete and persisting encapsulation

The epiphysis remains quite uniformly intact unless the joint cavity has been involved, erosions are then seen on the articular face The compact bone of the shaft has shown erosions on its surface secondary to a deep abscess in the overlying soft tissues Such a bone destruction in the shaft may give the appearance of a primary focus within the bone, until a projection at another angle proves it to be superficial I have not seen a case in which the attack on the compact bone from without has led to a penetration with the formation a medullary abscess

In an involvement of the short bones, which indeed is quite common, the picture is that of multiple foci, general osteoporosis, and early invasion of adjacent joints, with a minimum of bony proliferation

The usual appearance of well-marked necrosis and opposition seen in the ordinary lesions of the long bones is modified in those cases in which the clinical

course has been acute and in those which show a tendency to subside or heal The former show gross destruction and limited proliferation, while the latter show a much greater proportion of newly formed bone In an extremely rapid process the destruction may be complete, while the only evidence of opposition is seen on the shaft above the site of necrosis

The bone surrounding the ordinary blastomycetic focus presents a normal structure with little tendency to change in density This gives a rather sharp line of demarcation between bone apparently intact and regions of total necrosis Such a marked localizing destruction occurring within the spongy bone of a diaphysis together with a mature and homogeneous periosteal proliferation with or without cloaca, is so constantly present in the ordinary lesions of blastomycosis that when seen in further skiagraphs where the etiology has not been determined, a careful search for blastomyces should be made

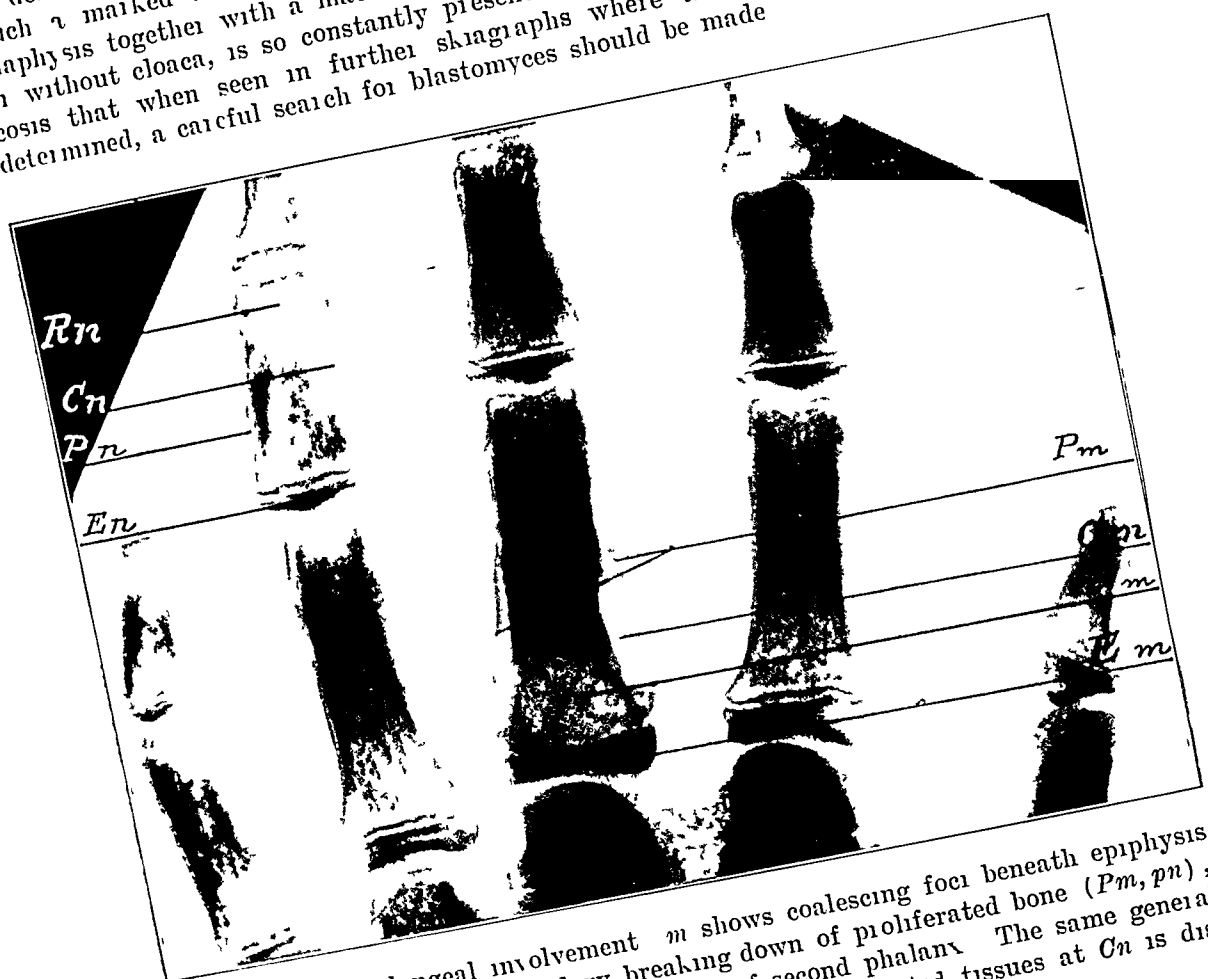


Fig 24 —Phalangeal involvement *m* shows coalescing foci beneath epiphysis (*Em*, *En*), *cm* shows a secondary breaking down of proliferated bone (*Pm*, *pn*), *Rn* a rarefication at a non epiphyseal end of second phalanx The same general characteristics are shown A softening of the proliferated tissues at *Cn* is distinct The adjacent joint is normal

The pathological changes found in the respiratory system of eight patients in this series conform in a general way to those described in the eight previous reports of post-mortem examinations Acute laryngitis and bronchitis were usually associated with active pulmonary lesions and the pleurae also presented changes in accord with those found in the lungs In cases with active pulmonary lesions, a serofibrinous pleuritis was quite a common finding and in one of our cases (Case 20) blastomycetes were isolated from the exudate In other cases

fibrous pleuritis was present, often obliterating the cavities The thickening of the pleurae at times was remarkable,[21] and the scars at the apices usually involved the adjacent tissues for some distance, in one case causing constriction of the jugular vein Pus in the pleurae occurred but once, this in a localized empyema found in Case 19 Fistulous passages in the pleurae extending to a lesion of the spinal column were found in one of our cases (Case 23) similar to that observed in Case 7, and communication with the left bronchus was noted in Case 22

A comparison of the lesions found in the lungs in these post-mortem examinations with those of eight previous reports, as mentioned before, suggests two methods of infection In a few cases the bronchogenic type predominated, but the majority showed diffuse hematogenous infection, the primary foci existing as fibrous areas about the larger bronchi

In the bronchogenic type much fibrous tissue existed, either solid or enclosing regions of infected lung tissue and usually located in the upper lobes

In one case an upper lobe was converted into a fibrous mass in which were areas of necrotic material, some of which were associated with cavity formation and dilatation of the bronchi[22] In marked contrast to such extensive fibrosis was the small area found in the lung of Case 21[23] This represented the only change in the lungs of a patient who a few months before had presented clinical evidences of wide-spread pulmonary infection The recent lesions consisted of small foci which have already been described as characteristic of blastomycosis, although in some cases distributed throughout the lobe or lung, they were quite discrete, and histologically such areas were often found unassociated with inflammatory changes in adjacent alveoli[24] This may explain the indefinite physical signs which were obtained in the examination of lungs which at necropsy were found to be extensively involved In other cases such foci had become confluent, forming larger areas of consolidation which were usually light gray[25] Softening in the center of such areas was occasionally present These focal lesions in some cases were distributed in such a manner as to suggest a bronchogenic origin[26] In other cases, however the wide-spread and uniform distribution closely resembled a miliary tuberculosis and could hardly have occurred except as a result of infection through the blood-stream In the more severe

21 Figure 7 article by Krost Moes and Stober, this issue
22 Figure 1, article by Myers and Stober, this issue
23 Figure 3 article by Lewison and Jackson, this issue
24 Figure 3 article by Churchill and Stober, this issue
25 Figure 3, article by Krost, Moes and Stober, this issue
26 Figure 1, article by Boughton and Clark, this issue

cases the infection was so marked that complete consolidation of the lung was produced, although here and there small nodules could be seen [27]

Concerning lesions in other viscera our observations also correspond to those in previous reports in that focal infections were quite common in the spleen, liver and kidney [28] Foci in the myocardium (Case 6), adrenal (Case 6), and pancreas (Case 5) had been found but once, and to this we are able to add instances of infection in the pericardium (Case 20) the adrenal (Case 26) and pancreas as well as four cases of blastomycotic abscesses of the prostate[29] (Cases 19, 20, 26 and 29), two of which were undoubtedly the source of the organisms found in the urine Nephritis was more or less marked in all cases, myocarditis in a few, and acute endocarditis in one case (Case 21)

While lesions elsewhere have been quite numerous it is worthy of note that involvement of the brain has previously been noted but once In the report of Case 7 a large abscess in the cerebellum together with lesions of the spinal dura and cord are described, and subsequently small lesions in the cerebrum were also discovered In the four cases of this series (Cases 19, 21, 22 and 26) in which complete examination of the brain could be made, local lesions were also found They consisted of small abscesses of variable size and location In cases 19, 22 and 26 they were found in both cerebrum[30] and cerebellum,[31] and in Case 21, one solitary nodule was found in the pons These have been discussed by Dr LeCount [32] The external cerebral dura was affected by extension of a bone abscess in Case 20, and the patient in Case 2 died of a meningitis, the blastomycotic nature of which was not determined Such a process, however, had been anticipated and was found in the necropsy in Case 27 by Dr LeCount The spinal cord also was involved in Cases 18 and 23

Of considerable interest is the existence of lymphadenitis in blastomycosis inasmuch as the slight tendency of the blastomycetes to invade the lymph-nodes has been dwelt on as a differential point between this infection and coccidioidal disease A general enlargement of the lymph-nodes was found at necropsy in about one-third of the cases, and blastomycotic lesions in addition to having been found in the peribronchial lymph-nodes a number of times were on two occasions in this series also found in the submaxillary, axillary and inguinal lymph-nodes

27 Figure 1 article by Churchill and Stober this issue
28 Figures 2 and 5 article by Myers and Stober this issue
29 Figure 2 article by Churchill and Stober this issue
30 Figure 6 article by Myers and Stober this issue
31 Figure 7 article by Myers and Stober this issue
32 LeCount E R Jour Nerv and Ment Dis 1909 xxxvi, 144

As a final supplement to the list and perhaps worthy of mention is the general infection of the eye which occurred in Case 20 Blastomycetes were obtained in pure culture from the vitreous humor, but no opportunity was afforded to study the pathological changes

In a majority of the cases in which a careful history could be obtained, the patients stated that their illness began with a cold or an acute infection of the respiratory tract, more or less intense in degree The more intense infections were sometimes preceded by a chill, and the symptoms most often noted were pain in the chest, fever, dyspnea, cough and expectoration of bloody and purulent mucus, to be followed sooner or later by the occurrence of the characteristic subcutaneous abscesses In other cases the onset was less abrupt and the initial symptoms slight and transient, the first manifestations to attract the patient's attention being the appearance of the local ulcerations or abscesses

After the disease became established the general symptoms were those of a chronic infection malaise, loss of strength, emaciation pain in the affected parts, irregular fever, rapid pulse and occasional chills and sweats Emaciation and loss of strength were usually proportionate In our cases the emaciation was extreme The face became drawn and haggard and assumed an expression which impressed the observers as being somewhat characteristic The enlargement of the affected joints presented a marked contrast to the emaciated extremities [33]

The temperature was most irregular, sometimes high, and at other times normal or subnormal In general, however, a fairly regular evening rise of temperature was present, and was usually associated with profuse perspiration In one case the high temperature occurred in the morning The pulse was usually increased in rapidity and often out of proportion to the temperature

Symptoms of pulmonary infection occurred sooner or later in all, and in some constituted the principal manifestation, although in no cases were the symptoms or physical findings commensurate to the extensive lesions found in the lungs at necropsy Cough was usually present, sometimes severe The sputum was thick and purulent or mucopurulent in character, often containing blood The latter was present in nearly all cases, but was irregular in occurrence and small in quantity No marked hemorrhages occurred Blastomycetes were present in the sputum in eight of the cases of this series, and in the mucus removed from the trachea at necropsy in the other two They were also noted in the sputum in four of the other reported cases

33 Figure 1, article by Wesson and Jackson, this issue

Their demonstration is quite easy when examined in 20 per cent solution of potassium hydroxid with a 1/8 inch objective (Fig 25)

Respirations were increased, and in a few cases dyspnea and hoarseness were present Sharp pains in the chest, due probably to the acute pleuritis, were not infrequent, but they rarely lasted more than a few days The majority of the patients complained of a dull aching sensation in the chest Of the physical signs, diminished expansion of the chest, due to fibrous pleuritis, was often found Dulness on percussion, the presence of râles, and increased transmission of breath and voice sounds were frequently noted, but as already stated, were not present in a degree proportionate to the changes found in the lungs at the post-mortem examination Areas of consolidation occurred first

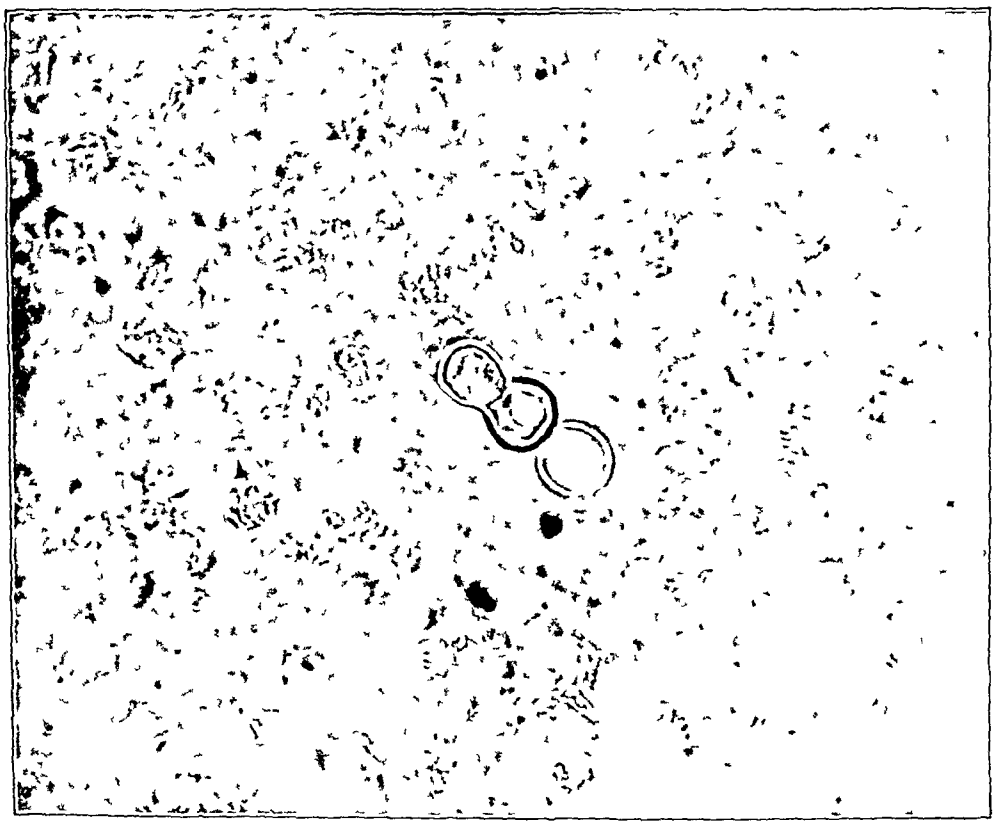


Fig 25—Round and budding forms of blastomycetes in sputum Specimen mounted in 20 per cent solution of sodium hydroxid (Negative by Dr W A Pusey)

as a rule in the apices or upper lobes of the lungs the right side being affected about three times as often as the left Râles over the bases with no definite signs of consolidation were not infrequent Very little gastro-intestinal disturbance was noted Anorexia was a common feature and diarrhea was present in five patients Blastomycetes were found twice in the feces The spleen was not constantly enlarged Nephritis was not uncommon and in several cases a marked edema occurred

Albumin and casts occurred in the urine, and blastomycetes, having origin in abscesses of the prostate, were found in the urinary sediment in two cases of this series

Pain was a constant factor in connection with the abscess formations, especially those involving the bones This was caused by the pressure of the enclosed pus, and relieved by incision and drainage The pain and tenderness in the affected joints at the onset were not so marked as in articular rheumatism, and not nearly so severe as would be expected with such extensive destruction as usually occurs later in the disease Insomnia because of the pain was a prominent feature, and without doubt caused the marked irritability and melancholia of some patients

Lesions of the spinal cord were responsible for paralysis of the lower extremities, and the involuntary discharge of urine and feces in two cases No symptoms were produced by the lesions in the cerebrum, but this could hardly be expected from their size or location One patient (Case 7) in whom a larger cerebellar abscess was found died during a convulsion, and signs of meningitis were manifested in one instance (Case 27) Marked neuralgic pain and gradual loss of vision, due to blastomycosis of the eye, were among the first symptoms noted in Case 20 The infection was located in the posterior chamber, and caused adhesions of the iris, irregularity of the pupil, opacity of the cornea, and marked congestion of the conjunctiva with purulent discharges

The blood showed a reduction in the number of red blood-corpuscles and the hemoglobin percentage that would be expected in a secondary anemia of such a chronic infection A leukocytosis is usually present, in some cases running as high as 30,000 per cubic millimeter The average number of leukocytes in the reported counts, excepting a few in which the influence of sepsis distorted the blood-picture, was 16,800 per cubic millimeter The average differential count was as follows polymorphonuclears, 73 per cent , small mononuclears, 17 per cent , large mononuclears, 6 3 per cent , transitionals, 0 7 per cent , eosinophils, 1 5 per cent , basophils, 0 1 per cent , myelocytes, 1 4 per cent In one case the myelocytes constituted 8 per cent The mononuclear elements are relatively increased This was more pronounced in those cases in which leukocytosis was less marked, as for example, one of the lowest counts (9,500 per cubic millimeter) showed only 55 per cent polymorphonuclears

The essential factor in the diagnosis of this disease is the demonstration of the blastomycetes in the sputum or in the pus of various lesions This can usually be done so easily that there would seem to be no excuse for not recognizing the disease when it exists

It should be remembered, however, that the organisms are easily overlooked in stained preparations, and the examination of fresh material is not a common routine measure It happens, therefore, that the organisms in most cases have been found only when sought for, and when the nature of the disease has been suggested by its clinical or pathological features

Blastomycosis should be ruled out in all cases of chronic pustular or ulcerative lesions of the skin, wasting pulmonary diseases, spontaneous superficial or deep abscesses, especially when connected with the bones, suppurative arthritis and suspected Pott's disease

DIFFERENTIAL DIAGNOSIS

The conditions with which blastomycosis has most often been confounded and the important differential points will be briefly enumerated

Coccidioidal Granuloma—This disease has been ably discussed in the articles by L Hektoen[1] and O S Ormsby[2] That the disease from a clinical and pathological point of view closely resembles blastomycosis is generally conceded The principal differential points advanced by the various authors are that in coccidioidal granuloma the nodular lesions resemble more closely those of tuberculosis, there is a greater tendency to involvement of the lymph-nodes, the cutaneous lesions are more ulcerative, the average duration of the disease is shorter than in blastomycosis, and reproduction of the organism in the tissues occurs principally by endosporulation

Epithelioma—The cutaneous lesions in Case 19 and also in other cases were first regarded as epithelioma, but close observation should readily differentiate these conditions The principal points are the slower growth of epithelioma, the greater induration—extending into the tissues beyond the ulcer margin, and the absence of the bluish-red halo surrounding the border, which contains the characteristic miliary abscesses, and in which the blastomycotic organisms are usually found

Tuberculosis—Reference has already been made to the close resemblance between blastomycosis and tuberculosis, both clinically and pathologically So far as the symptoms of the pulmonary infection are concerned, the clinical pictures of the two diseases are so similar that differential diagnosis is extremely difficult Tuberculosis is the more destructive of the two, and cavity formation and marked hemoptysis are therefore more common The examination of the sputum in most cases of course, gives positive evidence of the condition present, and blastomycetes should be looked for in those cases of suspected tuberculosis in which the tubercle bacilli are persistently absent

The affections of the bones are also very similar The small bones and joints are perhaps more often affected in blastomycosis the lesions

are more often multiple, and associated pulmonary, cutaneous and subcutaneous lesions are more common It should be noted that in several cases the blastomycotic ulcerations of the skin were mistaken for lupus A very interesting and important point emphasized in Case 23 was the marked simulation of Pott's disease by the blastomycotic lesion of the spine If any differences exist it might be stated that blastomycosis is more painful and rapid, and may begin by extension from the pleurae or adjacent structures rather than in the bodies of the vertebrae

The tuberculin reaction was tried in several of the cases in this series, with negative results

Syphilis—Some of the pustules and ulcerations might be mistaken for syphilitic lesions and the hard indurated character, with subsequent softening of the deep abscesses, is suggestive of gummata Roentgenograms of the affected bones have shown osteoporosis and periosteal thickening almost identical to that produced in syphilis, and one might be further mistaken and misled by the improvement which follows the administration of potassium iodid

Inasmuch as positive diagnosis of obscure cases of syphilis are now being made with the Wassermann serum test, and it being realized that this is a group reaction, it was deemed of interest to try the reaction with blastomycosis The serum in two cases was tested by Dr F G Harris One proved negative and the other was positive It was assumed that the latter patient was at one time affected with syphilis, although no history could be obtained The blastomycetic vaccine reaction may prove to be a positive factor in the diagnosis of blastomycosis In three cases of blastomycosis in which cutaneous lesions were present, a distinct inflammatory reaction was observed in the local lesions after the injection of the blastomycetic vaccine filtrate In the two cases which were not so far advanced a rise of temperature was obtained in from six to twenty-four hours after injection, the temperature in some instances persisting for two or three days Such a rise in temperature did not occur in two cases of syphilis, three cases of tuberculosis and three normal persons who were subjected to the test

Cutaneous tests consisting of the application of the filtrate and also triturated cultures in lanolin to scarified areas provoked no reaction sufficiently distinct to be considered specific Ophthalmic tests with the filtrate were also negative Different results might have been obtained with the use of cultures killed with less heat or in some other manner inasmuch as the ones used were subjected to a temperature of 110 C (230 F)

Dr Boughton, using suspensions prepared by Dr L Hektoen, noted the formation of small nodules at the site of puncture and inoculation

after the method of von Pirquet These did not occur in a subsequent test Other cutaneous and ophthalmic tests with these suspensions were negative

PROGNOSIS

Experience to date indicates that the prognosis is extremely grave, the mortality thus far constituting nearly 90 per cent It should be remembered, however, that in the majority of the cases the diagnosis was not established until the patients were in an extreme condition, and in several instances at necropsy The recovery in three cases (Cases 2, 13 and 24), the remarkable temporary improvement and the evidence of healed lesions in the lungs at necropsy in Case 21, and the healed and healing lesions of the lungs, pleura, peribronchial lymph-nodes and bones in Case 23 of this series, would indicate that the infection can be overcome by the system, and that with early diagnosis and proper treatment the mortality can be much reduced An important point to be remembered in connection with the prognosis given regarding local lesions is that live blastomycetes may persist for some time in the tissues of apparently healed regions of infection and again become active when treatment is discontinued or the patient's vitality reduced

The duration of the illness has varied from four months to two and a half years More than half the patients were sick for a period longer than one year In many of the cases death was due to secondary pyogenic infection

TREATMENT

Prophylaxis—If the previously mentioned theory of infection should prove to be true, prophylaxis could be rather easily established Pulmonary infection arising from the inhalation of mold spores could be prevented by the proper ventilation, lighting and drainage of residences and shops, thus preventing the dampness and moisture, which favor mold growth Cutaneous infection could also be avoided by the careful cleansing of wounds received in the presence of decaying or moldy wood or other vegetable material

Medical—The influence of food, climate and hygienic measures is undoubtedly as beneficial here as in any other chronic infection The recovery and cure in Case 13 was ascribed to climatic influence Of specific medication, potassium iodid in large doses seems to have the greatest effect It has been used successfully in a number of cutaneous infections and was given credit for the cure in Case 2 It was also administered for a time in Cases 13 and 24, mentioned before as cured, and in Cases 21 and 23 in which an improvement at least of the pulmonary condition was manifested (The internal administration of copper sulphate seemed to be of no benefit) For the cough

pain and insomnia, codein is especially valuable, although in a few instances morphin was necessary While medical treatment proved of benefit for the pulmonary infection, its influence on the metastatic lesions was not so marked, and these appear to be the cause of death in a number of instances In the histological examination of the bone and subcutaneous abscesses, one is impressed with the fact that the extension and increase in size of the infected areas is often directly due to the pressure of the growing fungi, and surgical interference would therefore be indicated to a greater extent than has been employed Clinically, excision of the cutaneous lesion has been very successful, and the relief of tension pain was always obtained by aspiration or drainage of the abscesses In a few cases healing of abscesses was affected by this alone Curettement of the bone abscess in Case 23 resulted in prompt healing, although reinfection occurred several months later

Solutions of copper sulphate and iodin were applied to the ulcers, but with indifferent results, probably for the reason that the solutions do not penetrate to a sufficient depth to reach the organisms Much better results were noted when these solutions were employed for irrigation of the abscesses The Roentgen rays have proved very beneficial in the treatment of the cutaneous lesions, but they were not used to any extent in the cases of this series

VACCINE THERAPY

Vaccination with blastomycetes and their products for therapeutic purposes was performed in three cases, two systemic cases (Cases 24 and 26) of this series, and one case of cutaneous infection (Fig 40) The latter was in the service of Dr O S Ormsby, through whose courtesy the treatment was instituted

The material used consisted of the filtrate and the suspension of the triturated membranes of bouillon cultures grown at room temperature for periods varying from two to six months The cultures were killed by heat (110 C) and preserved with 0 8 per cent chloretone Subcutaneous injection of either the filtrate or suspension produced an area of induration varying in size according to the quantity used A few of the larger areas became soft and fluctuating, owing to the formation of pus, which was sterile in all instances examined Such indurations and abscesses were more constant and pronounced when the suspension was used, and did not occur with small quantities of the filtrate *A certain amount of pain* was noted the first few days at the site of injection

The general effect of such injections was usually a rise in temperature of from one-half to two degrees[38] This became apparent in

38 Figure 4, article by Boughton and Stober, this issue

With the rise of temperature occurred a feeling of
id pain in the back and extremities Pain, however,
ly manifest in and about the local lesions, in which
iatory reaction occurred, consisting of pain, swelling
ge and often bleeding from the ulcerated surface
iom twelve to forty-eight hours The crusts became
s decreased in size and general improvement followed
proached the surface and ruptured, and healing of
ed These effects were quite as apparent from small
trates as from larger amounts of the emulsions The
ore general disturbance and temperature, in fact, a
re was noticed in three cases not blastomycetic in
esses followed the injection of 1 c c of the emulsion
mentioned above occurred most typically in Case 21.
ment began with the first injection, and was contin-
months the patient felt well and strong, and of
s which were present at the beginning of the treat-
remained and these were in an advanced stage of
nths later however one of these again became active
ous ulcer appeared Vaccinations were again resumed
at the lesions again are practically healed

wever, such good results were not obtained The patient
condition when treatment was instituted that a
could hardly have been expected On July 10 an
c c vaccine filtrate was followed by pain in the chest
gh, lasting for two days No increase of temperature
occurred Six days later an injection of 1 c c of
uced a rise in temperature of 1 8 F, but no pain
ocal lesions or in the chest On July 22 an injection
unt provoked only a slight rise in temperature, but a
in the superficial lesions was noted Slight burning
felt by the patient during the night, and the next
of the ulcers became soft and the crusts dropped off
g occurred for a few days, when the general condition
came much worse Cultures from pus aspirated from
esses at this time showed no blastomycetic growths,
ylococci and a few streptococci were present There
a few blastomycetes found in the sputum, and it is
te that nearly all were contained in giant-cells, a con-
s not previously observed

8, 2 c c of the filtrate were given, with very little
c c on August 21 produced no reaction whatever Dur-



Fig 26.—Case of cutaneous blastomycosis in which vaccines were used (Courtesy of O S Ormsby)

ing this time the patient had been gradually failing and death occurred six days later.

The case of cutaneous blastomycosis (Fig. 26) was under treatment for two months and at first some startling results were obtained. These, however, were only temporary. The patient was very anemic when he entered the hospital, the hemoglobin, estimated by the Dare instrument, amounting to 28 per cent. This was reduced by the repeated hemorrhages until only 12 per cent. was present. At this time the patient was unconscious and very weak. An arm-to-arm transfusion from a convalescing blastomycotic patient by Dr. Schroeder was followed by a rise to 27 per cent. and such an improvement in the patient's condition that consciousness returned, the dyspnea disappeared and he was able to sit up and partake of his food the following day. After a weak, however, he again became worse and died two days later.

From a most conservative point of view the limited experience with this method of treatment is very encouraging and suggests that the injection of the filtrate of old blastomycetic cultures may not only be useful in diagnosis but also prove to be a valuable therapeutic measure.

BIBLIOGRAPHY

(The numbers refer to the case reports in the order in which they were published. Cases marked * include necropsy report.)

*1 Busse: Centralbl. f. Bakt. u. Parasitenkunde, 1894, xvi, 175. Virchows Arch. f. path. Anat., 1896, cxlvi, 360. Buschke: Volkmann's Sammlung Klin. Vortr., 1898, No. 218. Bibliothek Medici, Abtheilung Dermatologie, Stuttgart, 1902.

2. Curtis: Ann. de l'Inst. Pasteur, 1896, x, 119.

*3. Montgomery, F. H.: Jour. Cutan. Dis., 1901, xix, 38. Walker, J. W., and Montgomery, F. H.: Further Report of a Previously Recorded Case of Blastomycosis of the Skin, Systemic Infection with Blastomyces, Death, Autopsy, Jour. Am. Med. Assn., 1902, xxxviii, 867.

4. Gilchrist: Brit. Med. Jour., 1902, ii, 1321.

*5. Ormsby, O. S., and Miller, H. M.: Jour. Cutan. Dis., 1903, xxi, 121. Otis, F. J., and Evans, Newton: The Morphology and Biology of the Parasite from a Case of Systemic Blastomycosis, Jour. Am. Med. Assn., 1903, xli, 1075.

*6. Cleary: Medicine, 1901, x, 818.

*7. Eisendrath, D. N., and Ormsby, O. S.: A Case of Systemic Blastomycosis, with Blastomycetes in the Sputum, Jour. Am. Med. Assn., 1905, xlv, 1015. Final Report by Le Count, E. R., and Myers, J., Jour. Infect. Dis., 1907, iv, 187.

*8. Bassoe: Jour. Infect. Dis., 1906, iii, 91.

*9. Irons, E. E., and Graham, E. A.: Jour. Infect. Dis., 1906, iii, 666.

10 and 11. Christensen, C., and Hektoen, L.: Two Cases of Generalized Blastomycosis, Jour. Am. Med. Assn., 1906, xlvii, 247.

12. Coley, W. B., and Tracey, H.: Jour. Med. Research, 1907, xvi, 237.

13. Herrick, J. B.: Generalized Blastomycosis, Jour. Am. Med. Assn., 1907, xlix, 328.

*14. Montgomery, F. H.: Jour. Cutan. Dis., 1907, xxv, 393.

15. Ormsby, O. S.:

16 Lyons, E E Cases 15 18 are included in the article on Systemic Blastomycosis by Montgomery, F H, and Ormsby, O S, THE ARCHIVES INT MED, August, 1908
17 Hyde and Montgomery
18 Oswald
*19 Krost, R A, Moes, M J, and Stober, A M
*20 Churchill, J, and Stober, A M
*21 Lewison, M, and Jackson, H
*22 Myers, H, and Stober, A M
*23 Boughton, T H, and Clark, S N
24 Boughton, T H, and Stober, A M
25 Jackson, H
*26 Bechtel, R E, and LeCount, E R
*27 Riley, F B, and LeCount, E R
*28 Eisenstaedt, J S, and Boughton, T H
29 Shaffner, P T

Hektoen, L Systemic Blastomycosis and Coccidiodal Granuloma, Jour Am Med Assn, 1907, xlix, 1071
Montgomery and Ormsby THE ARCHIVES INT MED, August, 1908
Hamburger, W M Jour Infect Dis, 1907, iv, 201
Ricketts, H T Jour Med Research, 1901, vi, No 3
Montgomery, F H Jour Am Med Assn, 1902, xxxviii, 1487
†Harter, André De la Blastomycose humaine, Nancy, France, 1909

†Since this article was completed a summary of the literature, together with a case report by Dr Harter, has been brought to our attention The bibliography of the work is most complete, especially the references to foreign literature

A CASE OF SYSTEMIC BLASTOMYCOSIS

R A KROSF, M D, AND A M SIOBER, M D
CHICAGO
AND
M J MOFS, M D
DUBUQUE IOWA

CASE XIX (1)[1]—*History*—M L, aged 42 married a native of Poland and laborer by occupation had lived in the United States four years He was admitted to the Cook County Hospital April 8, 1907 to the service of Dr Loeb with the diagnosis of acute articular rheumatism, because of the obvious swelling of the hand and foot

After admittance to the ward, the following history was obtained with the aid of an interpreter About four months before admission he was taken with a severe cold, cough and expectoration This interfered but little with his general health He next noticed a pain in the back at first felt only on motion, later when lying on the back A month later a swelling appeared at this painful area in the thoracic region at the left of the median line The cough which persisted from the onset became more marked, with abundant expectoration His appetite began to fail and he noticed that he was losing weight About four weeks before admission blood was present in the sputum for several days the general weakness became so pronounced that he was compelled to stop work six weeks after the onset Two months before admission painful swellings developed successively on the back of the left hand and foot and in the chest wall on the left side over the eleventh rib The next lesion appeared three weeks before admission as a small papule on the right ala of the nose This was quite painful and developed slowly into a warty growth A week later enlargement of the submaxillary lymph nodes on both sides of the neck was noticed He complained of cough, profuse expectoration night sweats marked general weakness and pain in the affected parts He denied previous illness of either a general or specific nature The family history was unimportant except that his first wife died of tuberculosis Three children born by his second wife are healthy The patient had been a coal heaver the past four years and his habits were good

Examination—The patient was a middle aged man, considerably emaciated, and very anemic He lay on the right side and suffered with much pain on even slight movements involving the affected parts The skin was moist and perspiration especially profuse on the forehead and in the axillae The respiration was free and rapid, averaging thirty per minute The pulse was full and regular the temperature 99 8 F, the scalp normal, the face drawn, the cheeks sunken, and the eyes partly closed The pupils were dilated, equal and reacted to light and accommodation There was no nystagmus or evidence of ocular paralysis In the middle of the forehead was a small papillomatous growth covered with a thin yellow crust On removal of this crust a small quantity of pus appeared The margin was indurated red and sharply demarcated from the surrounding skin At the margin of the right ala of the nose was another larger growth 1 5 cm in length The papillomatous character was more distinct, it extended

* Reported in part in Jour Am Med Assn, 1908, l, 184

1 Roman numerals in this and the following articles refer to number given in bibliography according to publication or occurrence Arabic numerals refer to number in this series

into the nostril for a distance of 0.5 cm., and was associated with considerable swelling of the adjacent tissues. The left ala of the nose was red, swollen and indurated, but the overlying skin was smooth. All these areas were very tender. The nasal cavity, mouth and pharynx were much congested with marked formation of mucus. No growths were visible, and the tonsils were not enlarged. The tongue was dry, the dorsum covered with a brown coat, the margins bright red. The teeth were in poor condition, many showing marked decay. In the submaxillary region on both sides of the neck was a hard, round, deep seated swelling about the size of a walnut. The anterior and posterior cervical lymph nodes presented a slight enlargement. No cervical rigidity was present. The thyroid was small and irregular. The bony framework of the chest was prominent, owing to the marked emaciation. Over the sixth rib on the left side in the nipple line was a rounded swelling about 2 inches in diameter. The overlying skin was red and adherent to the mass, and slight fluctuation was present. Posteriorly, on the right side over the twelfth rib, was another swelling of the same size, but the skin was not adherent and it did not fluctuate. Directly over the spine extending from the level of the eighth thoracic to the first lumbar vertebrae, and measuring 3 inches transversely, was still another swelling fluctuating and adherent to the overlying skin. Pressure over these areas excited marked pain.

Expansion of the chest was slightly retarded on the right side. On percussion the lungs showed no changes anteriorly. Posteriorly, dulness was obtained over the lower lobe of the left side and over a small area below the angle of the scapula on the right side. On auscultation of the lungs anteriorly a few bubbling râles were heard. On the left side over the upper part of the area of dulness the same signs were present, and whispered and voice sounds increased in intensity. At the base these râles were audible but the breath sounds were suppressed.

The heart was slightly enlarged to the left. The apex beat was palpable in the fifth interspace in the nipple line. A slight murmur could be heard over the apex, but it was not transmitted to the left, other sounds were normal.

The abdomen was scaphoid. On percussion and palpation no abnormalities were noted. The liver and spleen were not palpable. The genitalia were normal. The lymph nodes of the inguinal region were enlarged, forming a continuous chain on each side. On the dorsal surface of the right forearm were two small papillomatous growths, which resembled closely the one on the forehead. Considerable pain was elicited by deep pressure on the muscles of the forearm, but no swellings could be detected.

The left hand and foot were diffusely swollen, especially on the dorsal surfaces. The middle finger also showed a diffuse enlargement, being nearly twice as large as normal. These swellings were characterized by pain, fluctuation, tenderness and slight redness of the overlying skin. Examination of the nervous system revealed nothing abnormal.

Diagnosis, Treatment and Course.—The patient's condition aroused considerable interest and was diagnosed syphilis, pyemia and tuberculosis by different observers, the growth on the nose being generally regarded as epithelioma. Inasmuch as the diagnosis of syphilis was regarded as the most probable, the patient was given potassium iodid, 20 grains, and the protiodid of mercury, 1/5 grain, four times daily, with the effect of marked softening of the swellings. The potassium iodid was continued for a number of weeks, after which quinin and acetylsalicylic acid were administered.

On April 19 the character of the nasal growth was recognized and a diagnosis of generalized blastomycosis was made. Blastomycetes were then demonstrated in smears of pus from the nasal growth. The organisms were also found in the sputum, urine, sediment, and the aspirated contents of the swellings of the left hand and foot. The nasal growth was then excised and sectioned in celloidin. Microscopic examination showed changes typical of those described as occurring in blastomycosis of the skin. The epidermal layer presented a hyperplastic thickening with marked irregularity of the papillae. Scattered throughout were

numerous intra-epithelial abscesses densely packed with lymphocytes, desquamated epithelial cells and polymorphonuclear cells, the latter predominating Blastomycetes were present as round or oval refractile bodies singly or in clumps and budding forms were present No giant-cells were present in the purely intra-epithelial abscesses, but they were quite numerous in the cutis Here the cellular infiltration was dense, and except in a few areas the mononuclear cells predominated Many plasma cells and eosinophils were present In several places the blastomycetes were found in the giant cells Attempts to isolate the blastomycetes in pure culture resulted in failure because of the numerous other organisms present

Blood cultures at this time yielded the organisms 5 cc of blood from the median vein being distributed equally into ten large test tubes, each containing 10 cc of ordinary bouillon After incubation for three days two discrete white colonies were observed in one of the tubes suspended in the fibrin meshes which permeated the fluid These grew rapidly and began to show a distinct radiating structure From one of these a smaller colony gradually separated by its own weight and was later removed for examination and subculture to which reference will be made later[2] The removal of this colony which showed a distinct blastomycotic structure resulted in the dissemination of organisms in the bouillon for in a few days about thirty secondary colonies were observed scattered through the medium By this time the original colonies had attained a diameter of 5 mm the margins showing a fluffy radiating structure while the center was becoming clear and indistinct Pure cultures were easily obtained from the abscesses

April 24 the red blood cells numbered 2 420 000 per cubic millimeter the white cells 14,200 A differential count of 200 cells showed polymorphonuclear neutrophils 67 per cent large mononuclear leukocytes 9 per cent small mononuclear leukocytes 16 per cent eosinophils 2 per cent myelocytes 3 per cent and transitional forms 2 per cent May 8, the white cells numbered 22 800 per cubic millimeter Still another examination on May 16 gave a red cell count of 2 280 000 per cubic millimeter The hemoglobin was estimated at 70 per cent (Tallqvist) The leukocytes numbered 20,800 A differential count showed 77 per cent polymorphonuclear neutrophils 7 per cent large mononuclears 6 per cent small mononuclears 1 per cent eosinophils and 8 per cent myelocytes

On April 4 the urine possessed the following features color amber specific gravity 1 015 No sugar or albumin present Diazo reaction negative The sediment contained a few leukocytes No red blood cells or casts were present An examination on April 25 gave about the same results, except that the sediment contained a few hyalin casts and leukocytes and a large number of round and oval refractile bodies possessing a double contour and staining poorly with methylene blue On May 6 there was little change, except that the specific gravity was high, being 1 028 A careful study of the refractile bodies found in the sediment left no doubt as to their character as they did not dissolve in a 40 per cent solution of potassium hydroxid, and presented numerous budding forms They undoubtedly came from the pus from the abscesses in the prostate which were found at necropsy

The sputum, which was abundant was of a yellowish purulent character, with varying amounts of blood, which at times were sufficient to give it a brick red or chocolate color It was thick, but not tenacious Repeated examinations failed to demonstrate tubercle bacilli in it Many blastomycetes were found in the potassium hydroxid preparations Nothing of importance was found in several examinations of the feces

The patient became gradually worse and was helpless and bedridden after May 18 He complained bitterly of pain in the affected parts, especially on

2 This is the second case in which the organisms have been recovered from the blood, the first instance having been recorded by Buschke

motion, or when the abscess walls became tense from pressure of the enclosed pus Evacuation of these gave marked relief

The appetite continued fair, but in spite of this, he continued to lose flesh until the emaciation became pronounced No nausea or vomiting occurred at any time The bowels were fairly regular Urination was normal His sleep was often disturbed by the pain occasioned by involuntary movements about the bed Perspiration was profuse and constant His mental condition was very good, but became irritable and morose toward the end The cough was not especially marked at any time, but the expectoration was profuse The local lesions progressed more or less rapidly The induration about the nasal lesion excised April 19 extended into the nostril for some distance, and it is probable that not all of the infected tissue was removed, as the wound again took on a papillomatous appearance The opposite ala became red and swollen, and the surface covered with a thin brown crust The abscesses on the hand and foot became soft and were aspirated April 19 Marked relief from pain resulted for a few days, but it became necessary to incise and drain a few days later The pus obtained was thick and varied in color from a light yellow to a dark brown The abscesses on the back also were aspirated and the one on the left side of the chest incised May 15

During this time new abscesses formed in the right forearm and hand and in the inner part of the left thigh These were subsequently opened and pus of a similar nature evacuated With one exception, the discharge from these abscesses had been continuous and profuse, and a distinct moldy growth occurred on some of the dressings in which the discharge had become dried

There was a morning elevation of temperature, with marked evening remission, the latter often subnormal, at one time as low as 96 F The high points ranged between 100 and 102 F until the last two weeks, when temperatures between 102 and 103 8 were recorded The pulse continued fairly steady between 100 and 120 per minute until two weeks before the patient's death, when it began to follow the temperature curve, going as high as 140 During the last four days of his illness it varied between 120 and 160, the temperature being subnormal The respirations averaged about 26 per minute, and did not show any marked variations until the last week, when they also became more rapid During the last four days they were rapid and labored, from 54 to 60 per minute until the end Death occurred June 14 from exhaustion

Necropsy.—Permission for a necropsy was secured three days after death and the following anatomic diagnosis made miliary and nodular blastomycosis of the lungs, kidneys, spleen, cerebrum, pleura and lymph nodes Multiple blastomycotic abscesses of the cerebrum, cerebellum prostrate, pleura, skin, osseous muscular and subcutaneous tissues Parenchymatous nephritis Fatty changes, adenoma and angioma of the liver Adenoma of the thyroid General lymphatic hyperplasia Shaven beard appearance of Peyer's patches Atrophy of the testicles Fibrous pleuritis

The body was that of a middle aged man 177 cm (5 feet 10 inches) in length and extremely emaciated Considerable post-mortem lividity and some edema of the feet and ankles were present The surface of the body was marked by a number of fistulas and elevated swellings, from 2 to 8 cm (4/5 to 3 inches) in diameter and of three general types a distinct papillomatous growth with a raised surface occasionally covered by a thin brown crust, the margins indurated and surrounded by an areola of discoloration The second, excavated areas from 1 to 3 cm (2/5 to 1 inch) deep, with granular friable bases exuding a thin serum A third variety were lesions covered by thick brown rupia-like crusts removed with difficulty and resting on a bed of yellow pus The bases of the ulcers thus formed were much more granular than those of the second variety The last two varieties seemed to involve the subcutaneous tissues to a greater extent and suggested these as the point of origin These lesions were distributed in the skin

over the right upper lip right eyebrow knee elbow and costal margin the left cheek and on both alae of the nose

The fistulas were either open and discharging a thin yellow pus or were covered by a brown crust They were located on the inner side of the left foot, dorsal surface of the right hand and radial sides of both forearms All were the results of operation none of the abscesses having ruptured spontaneously The subcutaneous abscesses varied in size from 1 to 6 inches in diameter and were soft in consistency and distinctly fluctuating The overlying skin was pale usually, freely movable over the swelling and an edema of the adjacent soft parts was noticeable On incision a variable amount of thick pus escaped from a bright yellow to a reddish brown in color according to the amount of blood present The walls of the abscess cavities were smooth and thin Some were confined to the subcutaneous tissue and others communicated with cavities in muscle or bone Those confined more or less to the subcutaneous tissues were located as follows on the left side over the eleventh and twelfth ribs one measuring 5 cm (2 inches) in diameter It lay just underneath the skin to which it was adherent Another of about the same size was present at a point on the right of the spine opposite the fourth and fifth thoracic vertebrae Below this directly over the spine from the sixth thoracic to the third lumbar vertebrae and extending to either side a distance of 6 cm was a large fluctuating abscess It contained a large quantity of pus and extended deeply into the muscles exposing the seventh and eleventh ribs, and the ninth tenth, eleventh and twelfth thoracic and the first lumbar vertebrae the spine of the ninth thoracic being almost completely eroded, the others showing only slight involvement The muscles in the neighborhood had undergone pressure atrophy About an inch below this and to the left of the median line was another large fluctuating abscess 9 cm (3½ inches) in diameter

The abscesses in the muscular tissues seemed to be located between the muscles, separating them from each other and adjacent structures Some of these by extension were subcutaneous The walls of all were thin and smooth with a noticeable absence of ragged necrotic tissue After removal of the pus and cleaning some of the cavities it was almost impossible to locate the boundary In the submaxillary region was an abscess lying beneath the platysma and sternomastoid muscles It extended 6 cm (2½ inches) transversely and 4 cm (1½ inches) in a vertical direction Posteriorly the great vessels of the neck were exposed Another 20 cm (8 inches) in length and 6 cm (2½ inches) transversely was contained between the muscles of the thigh Its floor was formed by the muscles semitendinosus and semimembranosus separating the gracilis and the biceps The wall was made up of recognizable surrounding structures and it was covered by fascia and skin No communication with the bone existed In the right forearm on the radial side was a large swelling extending from the wrist to within 3 inches of the elbow, an abscess between the extensor communis digitorum and skin and the extensor carpi radialis brevis It had no distinct wall or communication with the bone

The abscesses involving bones were either confined to them or communicated with collections of pus in the adjacent soft parts

The sinuses on the dorsal surfaces of both hands led into small abscess cavities between the tendons and bones of the hands communicating with diffuse infiltration of the deep palmar tissues In the left hand one half of the shaft of the third and fourth metacarpal bones alone remained Slight erosion of the adjacent superior surface of the os magnum and the uniform was present On the middle of the left foot was a large abscess communicating with a cavity in the body of the internal cuneiform An incision into the right ankle revealed a diffuse infiltration of pus in the tissues about the joint with erosion of the internal malleolus The right knee joint contained turbid fluid and the synovial membrane was finely granular

Directly over the sternum was an abscess 2 inches in diameter which communicated through a small opening with an abscess in the bone This cavity measured ¾ inch in diameter and extended to the periosteum on the posterior surface (Fig 1)

The seventh rib on the right side about the middle of the shaft contained an abscess 2 cm (¾ inch) in diameter, communicating through a small opening in the periosteum on the internal surface with another collection of pus between the layers of the pleura This rib when split down the center to the costal end appeared normal to within an inch anterior to the abscess (Fig 2) From this point to almost the costal end the marrow was yellow The cancellous bone was softer than normal and the spaces were filled with a purulent material, which, on examination, showed many blastomycetes singly and in clumps

The deep cervical lymph nodes were enlarged and fused, forming continuous masses on either side, about 3 inches long, and an inch in diameter The majority were firm and light yellow On section small yellow nodular elevations 3 mm (⅛ inch) in diameter were seen Others were softer and contained calcareous masses The inguinal lymph nodes were also enlarged, the largest on the left side measuring 1 cm (½ inch) in diameter The submaxillary, superficial cervical and axillary lymph nodes were also somewhat enlarged, but did not show the nodules or softening Both pleural cavities were obliterated by fibrous adhesions, and the lungs were removed with difficulty

The left lung weighed 1,340 gm (about 3 pounds) The pleura was thin and was covered with numerous fibrous tags A number of miliary nodules could be seen and felt beneath its surface The lung was firm, did not collapse and crepitated feebly throughout On section, the cut surface was grayish black and diffusely infiltrated with small white or gray nodules from 1 to 4 mm (1/25 to 3/16 inch) in diameter The intervening pulmonary tissue seemed normal and on pressure exuded a clear frothy fluid Sections from the posterior portions where the nodules were confluent floated low in water Except for a small area, 1 cm (½ inch) in diameter, in the center of the lower lobe, no softening was present Considerable purulent mucus could be expressed from the bronchi, and in this a number of blastomycetes, some of which were budding, were found

The right pleural layers were united into a white fibrous coat most pronounced on the lower part of the lung, where it measured several millimeters in thickness Between its layers over the lower lobe and communicating with an opening in the periosteum of the seventh rib was the collection of pus already mentioned, occupying an irregular space measuring approximately 6 by 4 cm (2¼ by 1½ inch)

The right lung weighed 1 900 gm (4 pounds 3 ounces) It did not collapse and crepitated throughout On section (Fig 3) its appearance was very similar to that of the left lung, except that in the lower lobe the nodules were more confluent and formed irregular grayish white solid regions 1 cm (½ inch) or more in diameter No softening was present In the upper part of the lower lobe there was considerable fibrous tissue about the larger bronchi

The larynx, trachea and bronchi contained a large amount of frothy, purulent mucus The mucous membrane was smooth The peribronchial lymph nodes were enlarged and firm On section they were black and contained a few yellow regions from 1 to 3 mm (1/25 to ⅛ in) in diameter Others with no coal pigment were softer in consistency, translucent, and grayish red on section

The thyroid weighed 40 gm (1½ ounce) The left lobe was much larger than the right Little normal thyroid tissue was present the entire gland being made up of numerous round encapsulated regions from 0 5 to 2 cm (3/16 to ¾ inch) in diameter on section from gray to light brown

The pericardial cavity contained about 4 ounces of yellow fluid Its layers were normal The heart weighed 200 gm (7 ounces) and presented no gross changes The aorta had a few puckered areas of thickening in the arch The

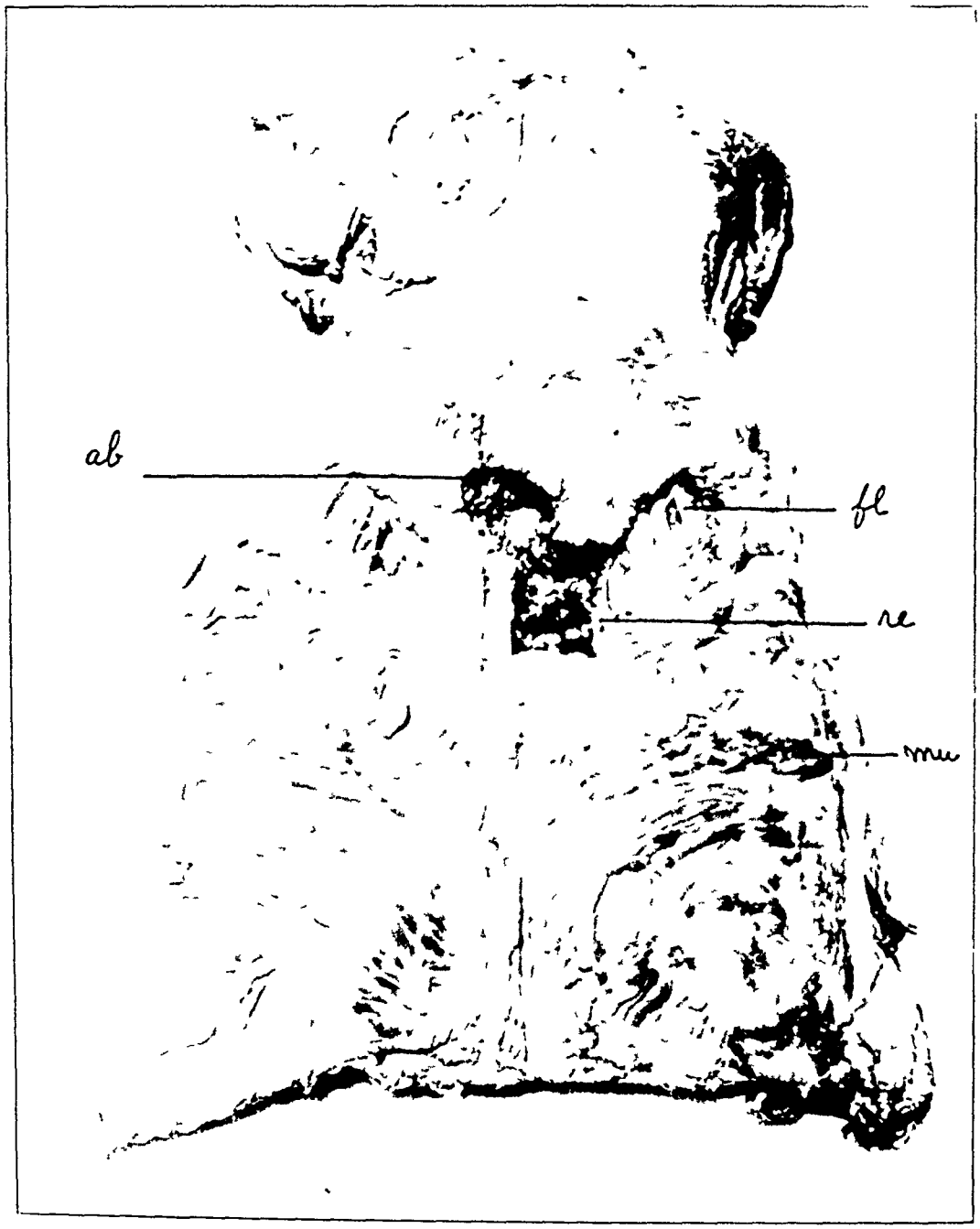


Fig 1—Blastomycotic abscess of the sternum *mu*, pectoralis muscle, *ab*, the opening into an abscess in the gladiolus, the extension of which can be seen in that area where a rectangular portion (*re*) has been cut away This lesion communicated with a large subcutaneous abscess the floor (*fl*) of which can be seen

periaortic lymph nodes were enlarged and grayish red, and contained small yellow nodules

The pharynx and esophagus were normal The tonsils were small and had large crypts containing a small amount of mucopus The peritoneal cavity contained a small amount of clear fluid The peritoneum was smooth Fibrous adhesions were present between the liver and colon, about the appendix and about the posterior margin of the spleen The omentum was devoid of fat A considerable degree of gastro enteroptosis was present

The stomach and pancreas showed only post mortem changes, the intestines the shaven-beard appearance of Peyer's patches

The gall-bladder and ducts were normal

The liver weighed 1,900 gm (4 pounds 3 ounces) The right lobe was enlarged and bore the imprint of the ribs The surface was smooth, yellow and

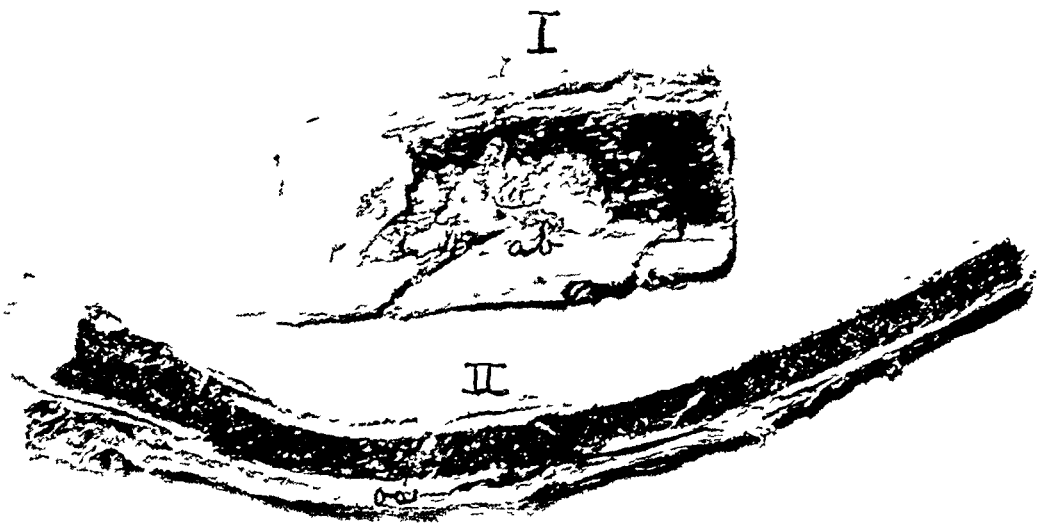


Fig 2—I, blastomycotic abscess (*ab*) of rib, part of which has been cut away Subcutaneous abscesses secondary to lesions of the ribs have been common features in most of the cases II, section of a rib showing blastomycotic osteomyelitis most marked at *os* The blood of this patient contained many myelocytes

mottled with red The centers of the lobules were dark-red, the peripheries light yellow In sections an angioma, 1 5 cm (⅝ inch) in diameter, and a small adenoma were found, but no nodules or abscesses were discovered

The spleen weighed 100 gm (3½ ounces) The capsule was somewhat thickened, the consistency firm On section the tissue was pale and the trabeculae and lymphoid tissue seemed increased Numerous blastomycetes were obtained from a few small yellow areas 1 mm (1/25 inch) in diameter

The kidneys together weighed 400 gm (14 ounces) On section of the left kidney the cortex measuring 8 mm (⅓ inch) rolled out beneath the capsule, the pyramids, from 16 to 18 mm (2/5 in) in length were dark The capsule stripped

readily leaving a smooth surface Beneath the capsule in the substance of the kidney were small yellow nodules from 1 to 2 mm (1/25 to 1/12 inch) in diameter quite solid and easily separated from the tissue When one of these was crushed under a cover glass in a 20 per cent solution of sodium hydroxid a number of blastomycetes were found The right kidney corresponded to the left except that the nodules described were more numerous twelve being counted on the cut surface and beneath the capsule The ureters were normal

The adrenals were normal except for a few small yellow nodules which were saved for histological examination The bladder was small the walls thickened and the trabeculae prominent The mucosa of the neck of the bladder and the posterior urethra were reddened and on pressure yellow pus escaped from the ducts

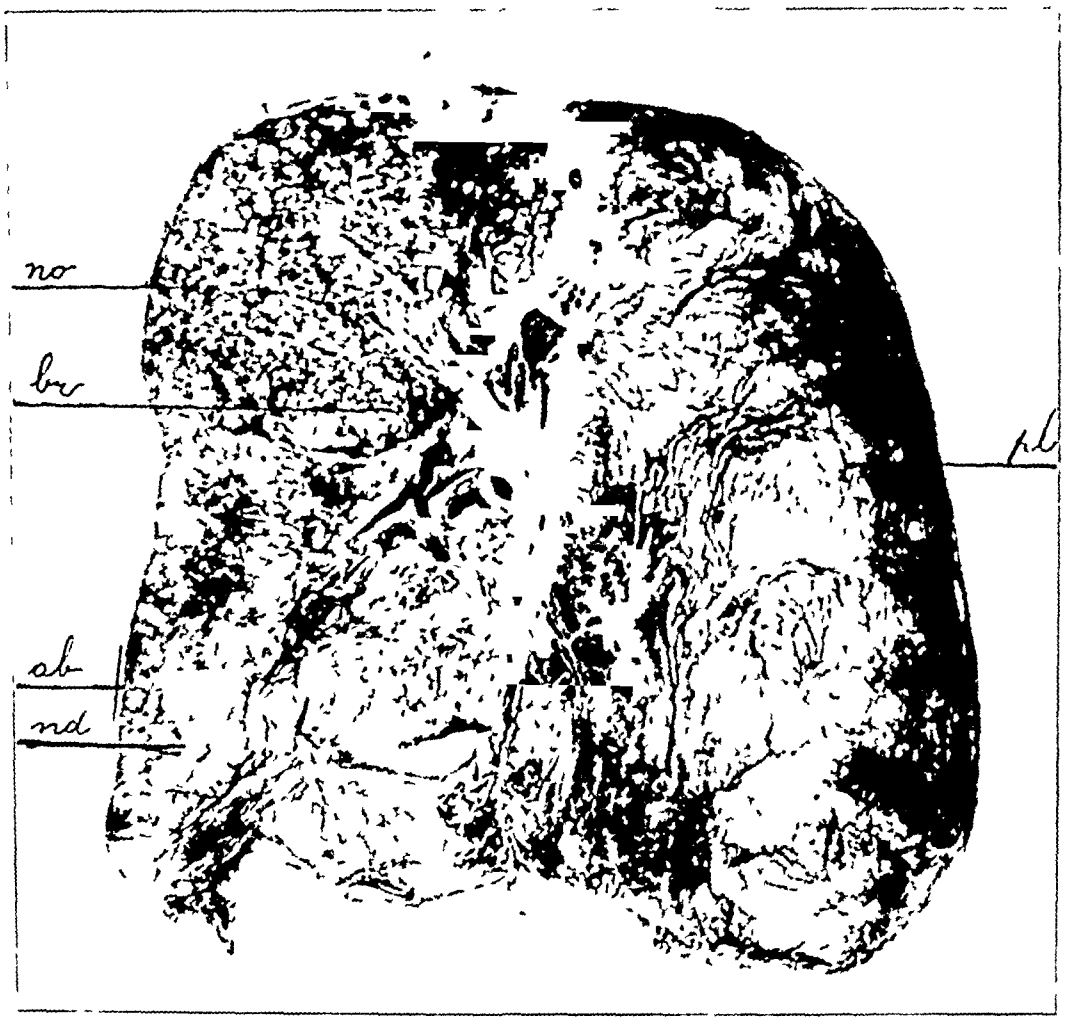


Fig 3—The right lung showing the thickened pleura (*pl*) and numerous nodes (*nd*) and nodules (*no*) on the cut surface A small abscess (*ab*) was present between the pleural layers, and considerable fibrosis existed about the larger bronchi (*br*)

The prostate was enlarged and contained a fluctuating region in the left lobe On section this lobe presented a central necrotic mass, with lines of the same material, or fine white nodules radiating to the capsule The right lobe contained a few small abscesses The seminal vesicles were distended with clear brown fluid The testicles were atrophic, the vasa deferentia normal

The brain weighed 1,400 gm (3 pounds, 1½ ounces) No gross lesions were present externally On section, in the cortex on the left side, near the middle of the sulcus between the parietal and occipital lobes, a small abscess 2 mm

(1/12 inch) was exposed Blastomycetes were demonstrated in the pus in this abscess In the right hemisphere a small nodule was found in the temporal lobe, one in the parietal lobe and two in the occipital lobe These were small, located in the cortex and usually at the margin of the white matter In the right lobe of the cerebellum there was also a small nodule, and a second occurred in the left lobe as a white nodular mass with central degeneration from 1 to 4 mm (1/25 to 1/6 inch) in diameter, this second lesion extended from near its internal surface to within ½ inch of the extreme lateral margin Blastomycetes were also demonstrated in the necrotic material from this area The spinal cord externally and on section presented no gross changes



Fig 4.—Section of the sternum showing infection of the bone marrow, 1, bone, 2, central zone of necrosis, 3, light areas representing clumps of blastomycetes, 4, peripheral border of unaffected marrow, 5, bony partitions which are sometimes absorbed, allowing the infected areas to become confluent, thus forming an abscess

The histological examination failed to reveal any lesions characteristic of blastomycosis in the liver, adrenal testes or vasa deferentia In the lungs there were found all degrees of coalescence of focal lesions, some so minute that they were little more than single alveoli, others large districts with more or less necrosis of their centers As a rule they possessed the feature of blastomycotic

nodular lesions elsewhere in the character of the cellular exudate giant cells organisms etc The fungi were very abundant In the thickened pleura covering the right lung the so called "miliary abscesses" were compressed and flattened out between the layers of fibrous tissue In the spleen isolated organisms, some of them budding were occasionally found in the sinuses at points where there was as yet no sign of tissue reaction at other places minute focal lesions had developed about them Many of the subcutaneous abscesses possessed no enveloping granulation tissue especially was this true when the wall was formed by striped muscle In the corium the inflammatory reaction was much more pronounced and evidences of pressure were abundant In some sections the fungi in the corium were so numerous as to resemble roughly fat areas with the high powers of the microscope

The lesions in the brain were peculiar in the abundant necrosis the appearance of bands of fibrous tissue between adjacent lesions probably due to pressure, and also in the angular bizarre giant cells which were relatively few The lining of the right knee joint was converted into granulation tissue resembling in a general way the margins of all large lesions organisms were present but not numerous The bone lesions differed perhaps most markedly from all the others in the comparatively little reaction of the tissue necrosis being the predominant change (Fig 3) In the prostate the change seemed confined to the glands which in the regions involved were more or less destroyed and converted into abscesses containing many organisms

Bacteriologic Examination.—In addition to the cultures obtained during life material was taken at necropsy from the knee joint and the abscesses of the spleen pleura and prostate Pure cultures were obtained from the first three mentioned and a mixed culture from the prostate The organisms were demonstrated in smears of material taken from the nodules of the kidney and the abscesses in the brain and rib All the cultures obtained from pus and necrotic material appeared to be similar, but that obtained from the blood showed a few differences A detailed comparative study of these is included with that of other organisms in the general summary

A CASE OF SYSTEMIC BLASTOMYCOSIS

T CHURCHILL, M D, AND A M STOBER, M D
CHICAGO

CASE XX (2) —*History*—J S, aged 39, a Polish laborer, was admitted May 14, 1907, to the service of Dr Heinick with the following history The patient states that his present trouble began three months ago with severe pain in the right half of the head and face, most marked, however, over the malar prominence This continued without remission until a month ago, at which time he began to experience pain in the right hip and knee The latter became much swollen, and so painful that walking or standing was prevented

A week later, pain was felt in the anterior part of the chest and in both shoulders, especially on inspiration or movements of the shoulders Two weeks ago the left wrist became tender and swollen A slight cough, present since the onset, has been more marked during the last two months, with blood occasionally in the sputum The power of sight in the right eye gradually diminished, and at present the patient cannot recognize small objects a few feet distant Although the appetite remained good he lost about 20 pounds He also states that previous to this illness he had been strong and healthy He is married and has five children Venereal infection is denied For the past four years he was engaged in the scrubbing and dusting of Pullman cars

Examination—The following points were noted in the examination The patient is of medium stature, extremely emaciated and appears to suffer much pain even on slight movement Beneath the scalp, over the vertex of the skull, is a tender fluctuating mass an inch in diameter The conjunctiva of the right eye is red and edematous Dulness is obtained by percussion over the upper lobe of the right lung anteriorly, and over the angle of the scapula posteriorly On auscultation tubular breath-sounds and bronchophony are heard over the apex in front, but no râles are present Over this dull area posteriorly the breath and voice sounds are diminished, but a few subcrepitant râles can be heard The left lung appears to be normal The heart is rapid but no changes can be demonstrated Nothing of importance was found in connection with the abdomen or its viscera

The right hip knee and sternoclavicular joints are red, swollen and painful on motion Fluctuation can be obtained over the knee The right great toe is also swollen, and contains an abscess which has ruptured, discharging thick blood stained pus Scattered rather symmetrically over the body are fourteen deep and subcutaneous abscesses, varying in size from 1 to 5 cm in diameter These are distributed as follows three in each arm, two over the chest and abdomen and six in the lower extremities There are also pustular cutaneous lesions distributed on the right side of the nose, right cheek, left arm and both legs

The temperature on admission was 101 F The subsequent course of the disease was characterized by morning remissions of temperature to 98 or 99 F and evening elevations to 101 or 102 associated with profuse sweats This continued until the last week of illness when the temperature became irregular and did not register much over 100 F until the day of the patient's death, June 20 The pulse varied between 90 and 120 beats per minute until the last ten days when it continued quite regularly between 120 and 140 per minute The respirations averaged 26 per minute, becoming somewhat more rapid during the height of the fever On June 15 a sudden change in the patient's condition was noted, the respirations becoming labored rising to 60 per minute He became cyanotic and complained of pain in the cardiac region, and for the next five days the respiration ranged between 44 and 56

Up to this time the patient had manifested but few symptoms Cough was of rare occurrence and expectoration was so scanty that material for examination was obtained with difficulty No organisms were found in that examined, but they were present in the mucus removed from the larynx at the necropsy No tubercle bacilli were found The abscesses increased in size and number, and were associated with great pain especially on motion A number of these were incised and thick bloody pus was evacuated

Diagnosis Treatment and Course—The diagnosis of blastomycosis was established June 10 by the demonstration of blastomycetes in the pus and their growth on culture mediums Prior to this the patient was given a preparation of iron quinin and strychnin as a tonic and morphin ¼ grain from one to four times a day for the pain On June 17 three days before death he was given potassium iodid 15 grains and copper sulphate ¼ grain three times a day without however any beneficial effect

The majority of the abscesses which were incised continued to discharge profusely and in the pus of a few of these the organisms could not be found A note relative to an examination made on June 17 of the condition of the eye is as follows 'The conjunctivae of both eyes are much congested and a purulent discharge is present The cornea of the right eye is opaque the pupil dilated and irregular and the iris clouded and adherent in its right lower quadrant The vessels leading to this region are engorged These results were corroborated by Dr Brown Pusey

Two crusted ulcers were present at this time over the left eyebrow three about the right shoulder two on the right knee three on the left knee and two on the left buttock, in addition to the lesions described previously, which had since become ulcerated A majority of these presented the papillomatous appearance characteristic of cutaneous blastomycosis It was noted that the abscesses which were not opened increased in size, and the left knee was also much swollen and fluctuation could be determined

Both jugular veins were dilated the left one measuring an inch in diameter the right forming a globular mass over 2 inches in diameter No pulsation could be determined, but the veins became much distended during inspiration The area of cardiac dulness was not much enlarged The heart sounds were obscured by the rapid respirations The lungs presented an impaired resonance in front with dulness to flatness behind On auscultation anteriorly the breath sounds were harsh in character and a few coarse râles could be heard at the apex On the sides the breathing was tubular in character becoming distant and indistinct posteriorly

A number of attempts to obtain cultures from the blood were made without success, and an estimation of the leukocytes made early in the course showed no increase No noteworthy changes in the urine were found The patient died of exhaustion as previously stated, on June 20, four months after the illness began

Post Mortem Examination—Through the courtesy of Dr W H Hunter, coroner's physician, the following data were obtained The body was extremely emaciated and presented the numerous cutaneous lesions and the superficial and deep abscesses previously described The latter were very extensive and contained a thick yellow or brown pus The walls were made of adjacent tissues, and no lining membrane could be observed, except in those cases in which the abscesses had been incised and secondary infection had taken place

An abscess 2 inches in diameter was present beneath the scalp, on the left side at the vertex It communicated with a region of softening in the parietal bone an inch in diameter involving almost its entire thickness An irregular perforation, 8 mm in diameter, existed in the inner table exposing the dura mater, the surface of which was rough and necrotic

The first rib contained an abscess near its junction with the sternum The bone here had been destroyed, and an opening in the periosteum communicated with a collection of pus beneath the bulging skin Similar abscesses were present in the third, fifth and sixth ribs on the right side The right knee joint was much

enlarged and its synovial cavity contained a large quantity of pus, the crucial ligaments were destroyed, and slight erosions existed at their points of attachment The articular surfaces were otherwise smooth The left knee also contained a large quantity of pus The left elbow was much swollen The olecranon had been fractured, and the head of the ulna was completely disorganized An abscess extended from this point into the forearm along the ulna to the wrist The terminal phalanx of the right great toe had been destroyed, and erosion with abscess formation in the small bones of the left wrist was present Still another collection of pus was found over the sternum communicating with an abscess in the sternum The latter occupied the greater portion of the right

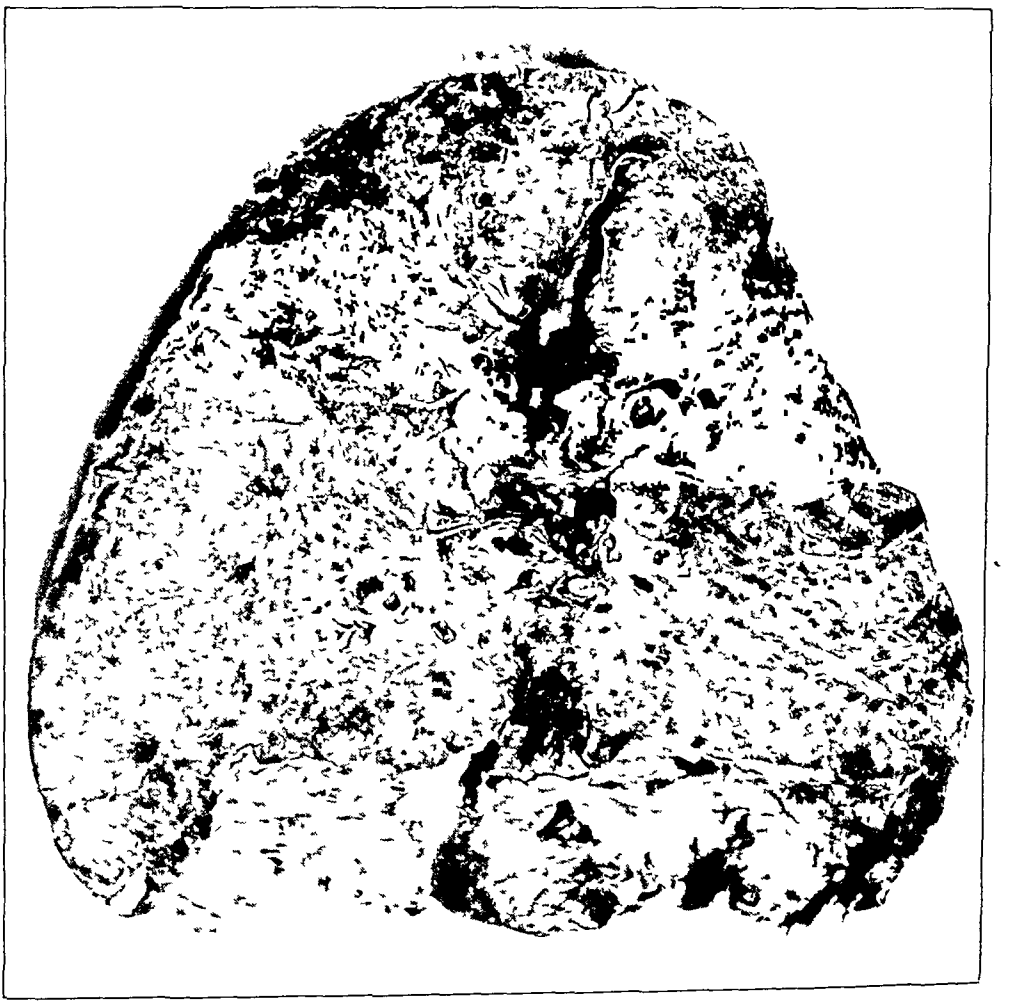


Fig 1.—Right lung The infection here was so extensive that the lung was almost solidified, and presented an appearance not unlike that of an organized pneumonia Blastomycotic nodules can be seen with difficulty, but the organisms were very numerous in the microscopic sections

half of the manubrium, and extended to the periosteum of the posterior surface On section the infected bone about the abscess was slightly yellow, and considerably softer than normal

The right eye showed the changes noted in the examination made a few days before death The tonsils were enlarged and the crypts contained a caseous material in which no blastomycetes were found The cervical, mediastinal and peribronchial lymph nodes were enlarged

Both pleural cavities were obliterated anteriorly by fibrous adhesions, which were especially firm at the apex (right) and opposite an abscess in the third rib The tissues about the apex were so involved in the cicatricial mass that the right jugular vein was constricted Above this point the vessel was dilated to the extent that its lumen measured 1½ inches in diameter Posteriorly, on both sides the chest contained a large amount of turbid fluid and the pleurae were covered with a thin fibrinous exudate

The right lung weighed 960 gm was quite firm and did not collapse Slight crepitation could be obtained over the greater portion The posterior surface was covered with a thick coat of fibrin and anteriorly fibrous tags were present The interlobar fissures were obliterated On section the upper lobe was firm, and dense white bands of connective tissue radiated from a cicatricial region torn from



Fig 2—Photomicrograph of blastomycotic abscesses of the prostate gland *ac* normal acini, *ab*, abscesses in infected acini *st*, fibrous stroma, *bl*, a small clump of blastomycetes

the third rib, to which it was adherent The intervening tissue was quite solid and contained very little air Numerous small white nodules were scattered throughout, and a few small caseous areas were present, the material from which contained many blastomycetes The middle and lower lobes on section showed an increase of the interlobular connective tissue The greater part of the lung was firm, smooth and translucent, with irregular intervening regions of apparently normal lung, from which a frothy fluid could be expressed No air was present in the solid portions Many small white nodules were scattered throughout the lung (Fig 1) The left lung weighed 700 gm , and presented a small amount

of fibrin on its posterior surface It crepitated feebly throughout, and on section the appearance was similar to that of the middle lobe of the right lung The general appearance was that of an organized pneumonia The small bronchi were filled with a thick purulent material in which blastomycetes were demonstrated The pericardial cavity contained 3 ounces of turbid fluid and the lining membrane was slightly roughened There was a slight thickening of the mitral valves No lesions were visible in numerous sections of the musculature

The stomach and intestines possessed no changes of importance, except that Peyer's patches were pigmented and reticulated The mesenteric lymph nodes were considerably enlarged The liver and kidneys were opaque and gray on cut sur

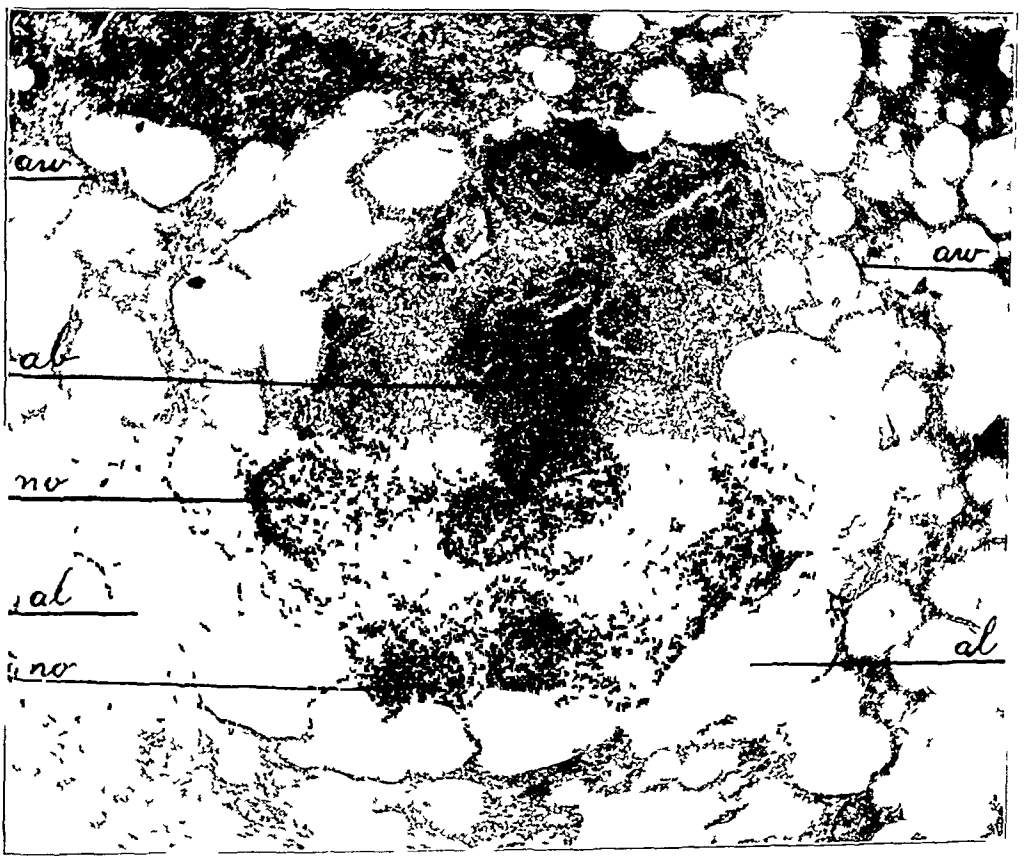


Fig 3 —Photomicrograph of a fairly typical blastomycotic lesion of the lung *ab*, necrotic center of a small node made up of *no*, confluent nodules, *al*, alveoli, *aw*, alveolar walls

faces The spleen was normal These viscera were carefully examined for blastomycotic lesions, and a number were found in the kidneys The adrenals, bladder, testicles, pancreas, vas deferens and seminal vesicles were normal The prostate was enlarged, and on section a small abscess 5 mm in diameter was exposed in the lobe The glandular structure of the left side showed slight changes

No nodules were found in the left lobe of the cerebrum, and the remainder of the brain was not obtained for examination

Anatomical Diagnosis—Serofibrinous pleuritis and pericarditis miliary blastomycotic nodules of the lungs, pleurae and kidneys, multiple blastomycotic abscesses of the lungs and prostate and the osseous, muscular and subcutaneous

tissues blastomycotic ulcers of the skin, blastomycosis of the right eye [1] purulent bronchitis, parenchymatous degeneration of the kidneys and liver, fibroid induration of the lungs, fibrous pleuritis

Histologic Examination.—No changes of importance were found in the stomach, intestine mesenteric or tracheobronchial lymph nodes The liver was moderately fatty in sections of the spleen occasional isolated blastomycetes were found without changes in the surrounding tissues In some of the kidney sections blastomycotic abscesses the size of a low power field (Obj III Ocular II, Leitz) occurred in the cortex and the usually rather abundant giant cells containing organisms were scattered about the abscess margins The heart muscle cells were atrophied and on the epicardium and beneath it the changes ordinarily present in slight fibrinous inflammations were found nothing however was found to indicate the essentially blastomycotic nature of the pericarditis [2]



Fig 4.—Photomicrograph of a section of thickened pleura *ft*, fibrous tissue *ab*, small abscess formations *gc* giant cells, *rc* areas of round cell infiltration

In sections from the sternum at some distance from the abscess, the changes were limited to the periosteum which was thickened and under which were col

1 Owing to the fact that the lesions of the right eye developed during the course of the illness, considerable speculation was aroused as to its nature Unfortunately permission to remove the eye was not obtained The eyeball was therefore rotated, a sterile puncture made and a portion of the vitreous humor removed From this, a pure culture of blastomycetes was obtained In view of this result the assumption that the lesion was one of metastatic blastomycosis is perhaps not far fetched Supporting this view are the facts that pain noted about the region of this eye and disturbance of vision, indicating its involvement, were among the first symptoms

2 See bacteriological examination

lections of partially necrotic leukocytes and many organisms The marrow appeared but little altered

In the sections of the cutaneous and subcutaneous lesions the changes in some were much like those described for chronic blastomycotic dermatitis, to which there were added the extensive regions of destruction produced by the deeper lying suppuration, in other sections the process was more limited to the subcutaneous tissues Except for the presence of the focal lesions so characteristic of this disease, which were scattered about the subcutaneous abscesses, the inflammatory process possessed the features of almost any chronic suppuration

In the prostate the great number of organisms filling some of the acini was indeed remarkable (Fig 2) The glands seemed dilated by the organisms alone From such accumulations accompanied by little alteration in the surrounding gland tissue there were all degrees of change for the extensive destruction noted in the gross examination Focal lesions, the minute blastomycotic "abscesses" with their peripherally located giant cells, were conspicuously absent in the prostate sections examined The changes in the lungs (Fig 3) varied in the different regions examined from recently produced bronchopneumonia to rather marked fibrosis In both, as well as in the greatly thickened pleura, the "abscesses" were scattered about, although in the pleura these disseminated lesions with organisms were flattened out between the dense masses of fibrous tissue (Fig 4)

Bacteriology—Pure cultures of blastomycetes were obtained from the pus of various abscesses, from time to time during life At the post mortem examination, medium was inoculated or material taken for inoculation from the blood, bile, pleural, pericardial and peritoneal fluids, the vitreous humor of the right eye and pus from the knee joint, and the abscesses of the neck and prostate gland

Streptococci were grown from the blood, pleural and peritoneal fluids and the pus of the knee joint The blastomycetes were isolated culturally from the pericardial and pleural fluids vitreous humor of the eye and the prostatic abscess The results of study of these organisms are mentioned elsewhere in this report The presence of the streptococci was regarded as the result of a terminal infection inasmuch as there were clinical manifestations of such a condition during the few days prior to the patient's death The failure to recover any organisms culturally from the pus of the neck and the knee joint would indicate possibly that with and without secondary infection the blastomycetes may die or disappear from the pus of the abscesses in which they had previously been demonstrated

Thanks are hereby extended to Dr Heinick, attending physician, for his permission to report the case

A CASE OF SYSTEMIC BLASTOMYCOSIS

M LEWISON, M D AND H JACKSON, M D
CHICAGO

CASE XXI (3) —*History*—M C Italian aged 17 was admitted to the Cook County Hospital May 20, 1907, to the service of Dr Goodkind with the following history His illness began five months ago with the appearance on the anterior surface of the middle of the right thigh of an abscess which ruptured and discharged a thick fetid dark brown pus A papillomatous crust formed subsequently at the site of the abscess There was at the same time a small abscess near this which healed in a few days Two months later a sharp stabbing pain occurred in the right knee Motion soon became restricted and in two weeks a swelling of the joint appeared This gradually increased so that in two months the knee was twice its normal size It remained in this condition until the time of his admission to the hospital One month after the onset of the pain in the right knee the left knee became similarly involved but the pain and swelling were less severe The right ankle also became affected but the process did not progress and at present (the time of his admission) shows no signs of inflammation Marked swelling accompanied by pain and tenderness occurred successively in the left ankle both elbows left wrist and the first metacarpophalangeal joint of the left hand A small papule developed in the left eyebrow one month after the commencement of the joint troubles and since then similar lesions have appeared on other parts of the face and head (Fig 1)

The patient was born in Italy and from the age of 10 was engaged in hard manual labor on a farm Four years ago he came to the United States and has worked at intervals as an organ grinder and helper in a machine shop until the onset of the present illness He lived in the congested Italian district on the west side of Chicago and his living quarters were unhygienic in the extreme The rooms themselves were dry at the time of investigation (summer) but the basement in which the closets were located was wet and decomposing feces and wood were found in many places His health had always been good and he denied venereal infection The family history was unimportant except that his father died two years ago after an illness of two months during which time he coughed frequently, often expectorating blood

First Admittance to Hospital—When admitted to the hospital, on account of the multiple arthritis, the diagnosis of chronic rheumatism was made The course of the disease, however, during the subsequent two months led to the consideration of tuberculosis and syphilis as possible explanations of the symptoms, and the latter was favored because of an interpretation given to the appearance of the bones of a roentgenogram of the hand The subsequent development of an abscess beneath the scalp, and its characteristics resembling those encountered in other cases of blastomycosis, led to the examination of aspirated pus and the discovery of the organisms The patient now was anemic and emaciated, seemed quite comfortable and complained of pain and stiffness of the joints The pulse was 108, temperature 101 8 F , respirations 28 On the face were a number of small oval, indurated purplish ulcers, their edges being fairly regular and rounded margins sloping outward to the skin around them The largest of these situated above the right eye and measured 6 by 20 mm Other similar lesions in various stages of involution were present on the back of the neck, scalp, arms, thighs and left ankle An oval "rice paper" scar was present on the upper part of the right thigh, and below this a fistulous opening discharged bloody pus It was surrounded by an oval area of indolent

granulation tissue and represented the site of rupture of the first abscess which appeared Beneath the scalp three small abscesses were found, one of these was more deeply situated and seemed to be connected with the bone

The eyes, ears, nose and neck were normal The chest was negative on inspection except over the tenth rib in the posterior axillary line on the left side, where there was a soft rounded swelling the size of a hazelnut, and evidently attached to the rib Resonance was impaired over both apices in front and



Fig 1—M C, photographed a few days before death Note the cutaneous lesions on the left cheek and right hand, the swollen and ulcerated condition of the left elbow, and the marked swelling of the knees

behind The respiratory murmur was roughened and expiration prolonged No rales were heard anywhere Posteriorly below the left scapula, resonance was impaired and the voice and breath sounds slightly diminished A faint systolic murmur over the pulmonic area and a slight enlargement of the liver were the only alterations in the further examination of the chest, abdomen and genitalia

The muscles of the extremities were atrophied Both elbows, the right wrist, both knees and the left ankle were swollen and tender, and motion was restricted and painful There were in the right hand two small abscesses involving the first metacarpophalangeal joint and the middle phalanx of the fourth finger A sinus on the palmar surface of the fourth finger communicated with the last-named abscess and discharged a thick brown pus The left wrist was tender and extending from an abscess in the first metacarpophalangeal joint were two sinuses one dorsal and one palmar each discharging a thick yellow pus

There were no disturbances referable to the nervous system The superficial cervical and epitrochlear lymph nodes were slightly enlarged

The urine was amber clear acid in reaction specific gravity 1 018 and contained no albumin sugar or casts No blastomycetes were found in the urine obtained after massage of the prostate Examination of the feces failed to show anything of importance

The blood contained 3 800 000 red cells per cubic millimeter 9 600 white cells and 65 per cent hemoglobin (von Fleischl) A stained specimen showed slight poikilocytosis but no nucleated red cells The differential white count was as follows polymorphonuclear neutrophils 55 per cent small lymphocytes 32 per cent large lymphocytes 11 per cent , eosinophils 2 per cent No cultures were obtained from the blood in several attempts

The course of the disease was slow and marked by several remissions and exacerbations The pulse ranged from 80 to 120, the temperature was highest in the evening and varied between 98 and 103 6 F After June 22, when the diagnosis was established the patient was given increasing doses of potassium iodid, tonics and fresh air treatment and rapidly improved The pain in the joints disappeared and he left the hospital July 8 and resumed his occupation organ grinder

Second Admittance to Hospital—Ten days later he returned to the hospital with a marked aggravation of all his joint symptoms He coughed much expectorating slight amounts of mucoid material and suffered greatly from pain in the joints, the pulse was 84, temperature 100 8, respirations 24 The right ankle which was normal previously was swollen and tender, and two new abscesses had appeared on the ulnar side of the right wrist and on the dorsal surface of the left wrist extending into the hand

The physical signs in the lungs were also more pronounced, but no rales were heard Administration of potassium iodid was resumed with no improvement for about eight days Many new abscesses appeared successively on the hands and feet, and the temperature ranged up to 103 4 F Copper sulphate was now given internally in ¼ grain doses three times a day and the hands and feet, in which were many discharging abscesses were dressed with hot 0 5 per cent solution of copper sulphate The patient again improved, the cough subsided, the abscesses healed and the joints became less painful

Repeated examinations of the sputum were made for the tubercle bacillus and blastomycetes with negative results until August 5, when budding blastomycetes were found The improvement continued and the patient gained in weight and strength The temperature did not rise above 99 F after August 11 All the fistulas closed and he left the hospital August 17 in good condition, there being only slight limitation of motion in the elbows and knees At the time of discharge 150 grains of potassium iodid three times a day and ½ grain of copper sulphate three times a day were being given

Third Admittance to Hospital—The patient again applied for admission to the hospital Dec 12, 1907, and was assigned to the service of Dr Mix and afterward transferred to the surgical service of Dr Ryerson During the second furlough of four months he suffered many hardships He had taken no medicine, and had tried to return to Italy, but the officials at New York refused him passage and he was forced to sell papers on the streets in order to obtain food He entered the hospital much emaciated and in a very weak condition,

with an exacerbation of all his old lesions and some new ones The pulse ranged from 90 to 130, temperature 98 6 to 103 6 F, respirations 22 to 30 There was a general adenopathy, especially noticeable in the axillary, cervical and inguinal regions Large abscesses involving both knees, wrists, elbows and ankles were present, the one about the right knee being very large and fluctuating and extending half way up the thigh The others had ruptured and discharged a thick yellow pus He suffered excruciating pain on movement of any of these joints The ulcers of the skin were larger, and new lesions were present on the lip and the back of the neck Impaired resonance and harsh breathing obtained over the apices of both lungs

Increasing doses of potassium iodid and a nutritious diet were again administered, but the patient became weaker despite treatment and died Jan 16, 1908

Early in the course of the disease, Bier's passive congestion treatment was used, but it seemed to do little good As soon as the patient was able to leave his bed he spent nine hours a day in the sunshine and open air

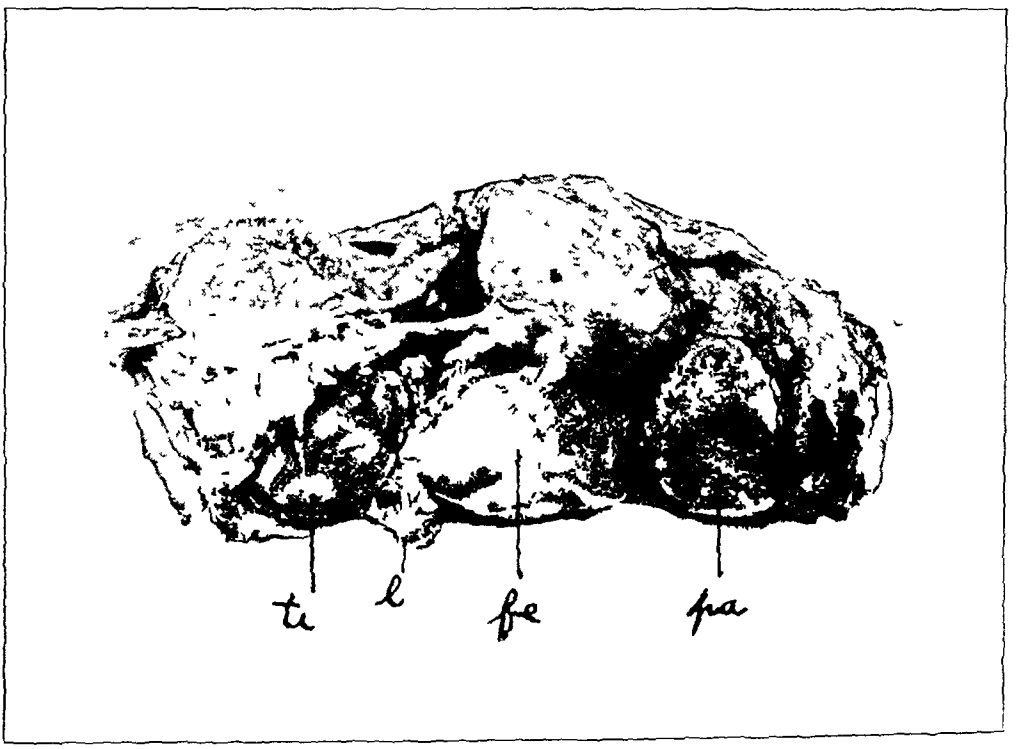


Fig 2.—Articular surfaces of left knee *ti*, eroded surface of head of tibia, *fe*, lower end of femur on which only a portion of the articular cartilage remains, *pa*, patella, in which are two abscesses, the openings of which are exposed, *l*, articular ligaments which were so disorganized that subluxation occurred

Necropsy.—A post mortem examination (Dr Stober) was held two days after death and the following is the anatomical diagnosis indurated blastomycotic bronchopneumonia of the left lung blastomycosis of bones, subcutaneous and cutaneous tissues pons Varioli inguinal and axillary lymph-nodes, acute vegetative mitral endocarditis fatty liver, parenchymatous degeneration of the kidneys, fibrous pleuritis, hyperplasia of the lymphoid tissue of the ileum, edema of the lungs, hydropericardium, atrophy of the heart, pancreas and testicles, emaciation and anemia

The body is that of an extremely emaciated young man Numerous cutaneous lesions abscesses and fistulas are present, the majority of which have been described in the clinical report Both knees are markedly swollen and the

skin covering them contains several ulcers and discharging fistulas A posterior subluxation has occurred in the right knee and the same condition can be produced in the left knee The joint cavities contain a large amount of bloody pus, the periarticular tissues are disorganized, the ligaments destroyed and the cartilages have disappeared exposing the rough granular surface of the bones (Fig 2) About these joints large communicating abscesses exist, the one above the left knee extends the whole length of the thigh and contains nearly a quart of pus Another above the right knee is about half as large Discharging abscesses involving the elbows and the small bones of the feet are also present The articular structures of the latter have been practically destroyed There are abscesses communicating with the lymph nodes in both groins and in the right axilla The lymph nodes of the left axilla are enlarged and pink on section

The pleural cavities contain about 2 ounces of a clear yellow fluid, with slight fibrous adhesions about the apices and also to the diaphragm on the left side The pericardial cavity contains 1 ounces of a clear yellow fluid

The lungs weigh 620 gm Both crepitate freely and do not collapse The left lung on section is mottled light gray and on pressure exudes a slight amount of frothy fluid In the extreme lower margin of the lower lobe and externally is a region of induration roughly 5 by 1 cm in diameter somewhat circular in outline and directed toward the root of the lung where it is continuous with a similarly indurated tissue behind the main bronchus in which there are several yellow regions of necrosis (Fig 3) The pleurae overlying the consolidated lung tissue near the diaphragm is thickened and binds the lung to that structure The lung beneath contains numerous small blood vessels and bronchi surrounded by firm tissue heavily pigmented with coal dust The pericardium is generally adherent to the left lung The remainder of this lung as well as the right lung is unchanged The tracheobronchial lymph nodes contain large amounts of coal pigment and are slightly enlarged

The tongue is normal The tonsils are small and from their crypts a purulent material is expressed The esophagus is smooth the trachea and larynx are slightly hyperemic and contain mucopus

The heart weighs 240 gm The pulmonary tricuspid and aortic valves are normal The mitral valve is thickened and on the edge of both cusps are fine warty vegetations the largest of which measures 1 mm in height The heart muscle is pale the left ventricle wall measures 5 mm in thickness, the right 2 mm The aorta and coronary vessels are normal The liver weighs 1,400 gm Its surface shows the imprint of the ribs On section the tissue appears somewhat opaque and light yellow The spleen weighs 200 gm Its capsule is thickened, its consistency firm, the cut section brown and there is an increase in the connective tissue No microscopic nodules are present

The brain weighs 1 600 gm It is normal externally In numerous sections one blastomycotic nodule is found in the pons a few millimeters below the ventral surface

The kidneys weigh 280 gm On section the kidney substance bulges, it is a grayish yellow, the cortex is increased in thickness and the capsule strips readily, leaving a smooth surface

The testicles are small, and sections expose an increase in connective tissue The bladder is small and contains a small amount of turbid urine The rugae are fairly prominent, the posterior urethra is congested and slightly granular The prostate and seminal vesicles are normal The pancreas is firm and small The mucous membrane of the stomach is thin, and small ecchymoses are present about the pyloric region The intestines are normal

The left leg was amputated through the thigh for a more detailed examination A small opening into the knee joint from an abscess in the internal condyle was found, as well as several small regions of softening in the cancellous bone of the patella The abscess in the condyle was 3 cm in diameter and the passage into the joint one third as large The bone about the

abscess as well as that just beneath the articular cartilages of both tibia and femur was soft and diffusely yellow, on crushing a semipurulent fluid was obtained (Fig 4) On the antero internal surface of the tibia at the junction of the upper and middle thirds an opening 5 mm in diameter was found passing through a raised bony and shell like periosteum under which was a small abscess not connected with the marrow cavity The epiphyses of the lower end

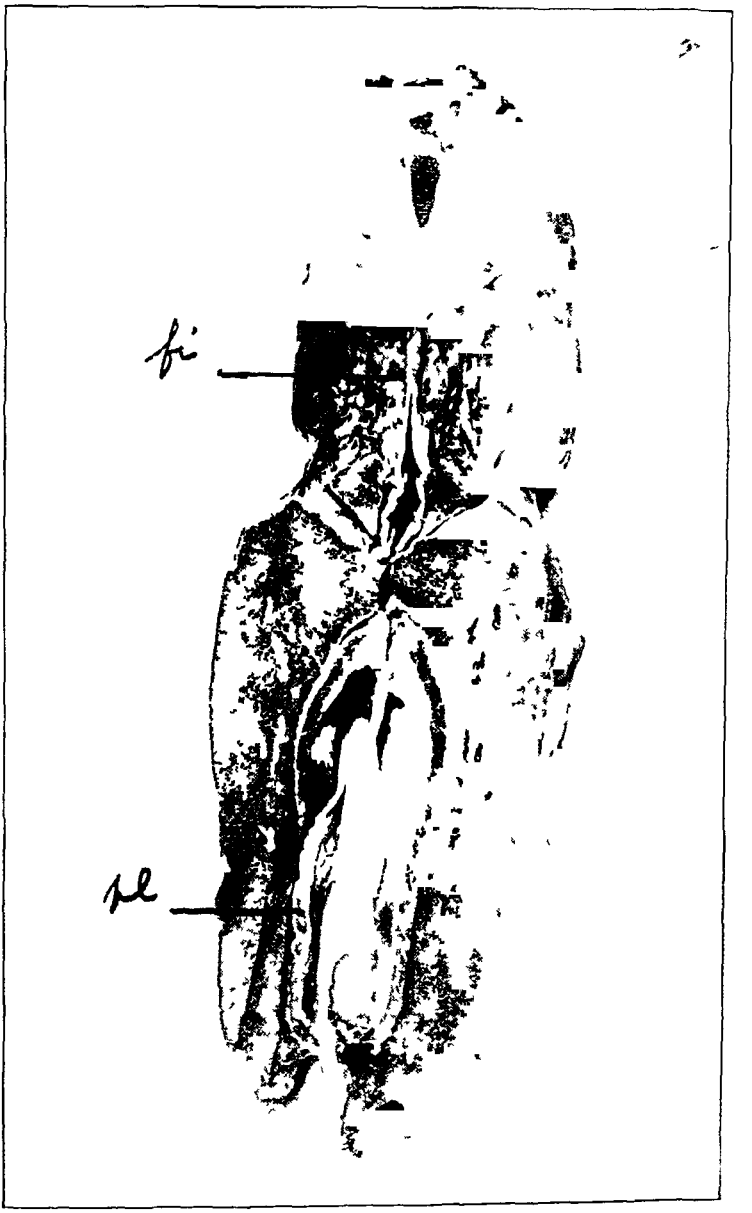


Fig 3—Section through the lower lobe of left lung *fi*, fibroid area representing healing blastomycotic lesions, *pl*, thickened pleura adherent to the diaphragm

of the tibia and fibula also contained small yellow softened regions communicating with abscesses over both malleoli which had ruptured externally (Fig 5), the one over the external malleolus continuous with an abscess extending beneath the Achilles tendon Occupying the greater part of the dorsum of the foot was a sharply demarcated region of necrosis, and the tissues bordering this were

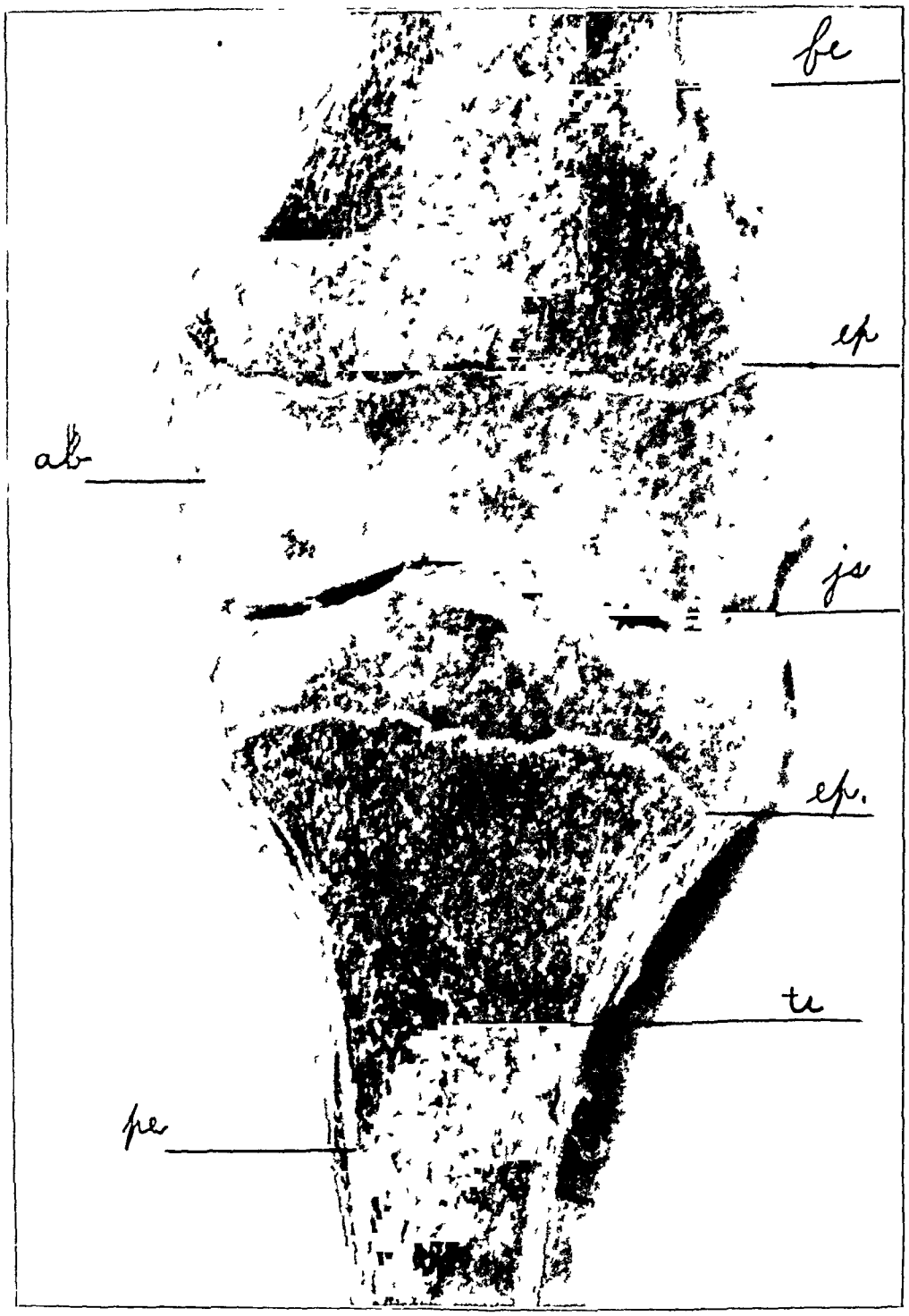


Fig 4 —A longitudinal section of the left knee joint *fe*, lower end of femur, *ti*, upper end of tibia, *ep*, epiphyseal line, *js*, eroded joint surfaces, the light color of the adjacent bone represents the extent of the blastomycotic process, *pe*, healed periostitis, *ab*, abscess in epiphysis which communicated with the joint

without evidence of reaction In this region the unchanged extensor tendons lay bare, and beneath were the fragments resulting from the disorganization of several of the tarsal and portions of the metatarsal bones (Fig 6) In the pus of the joint cavities, bone abscesses and adjacent soft cancellous marrow were to be found many round and budding blastomycetes, some of which possessed capsules of unusual thickness

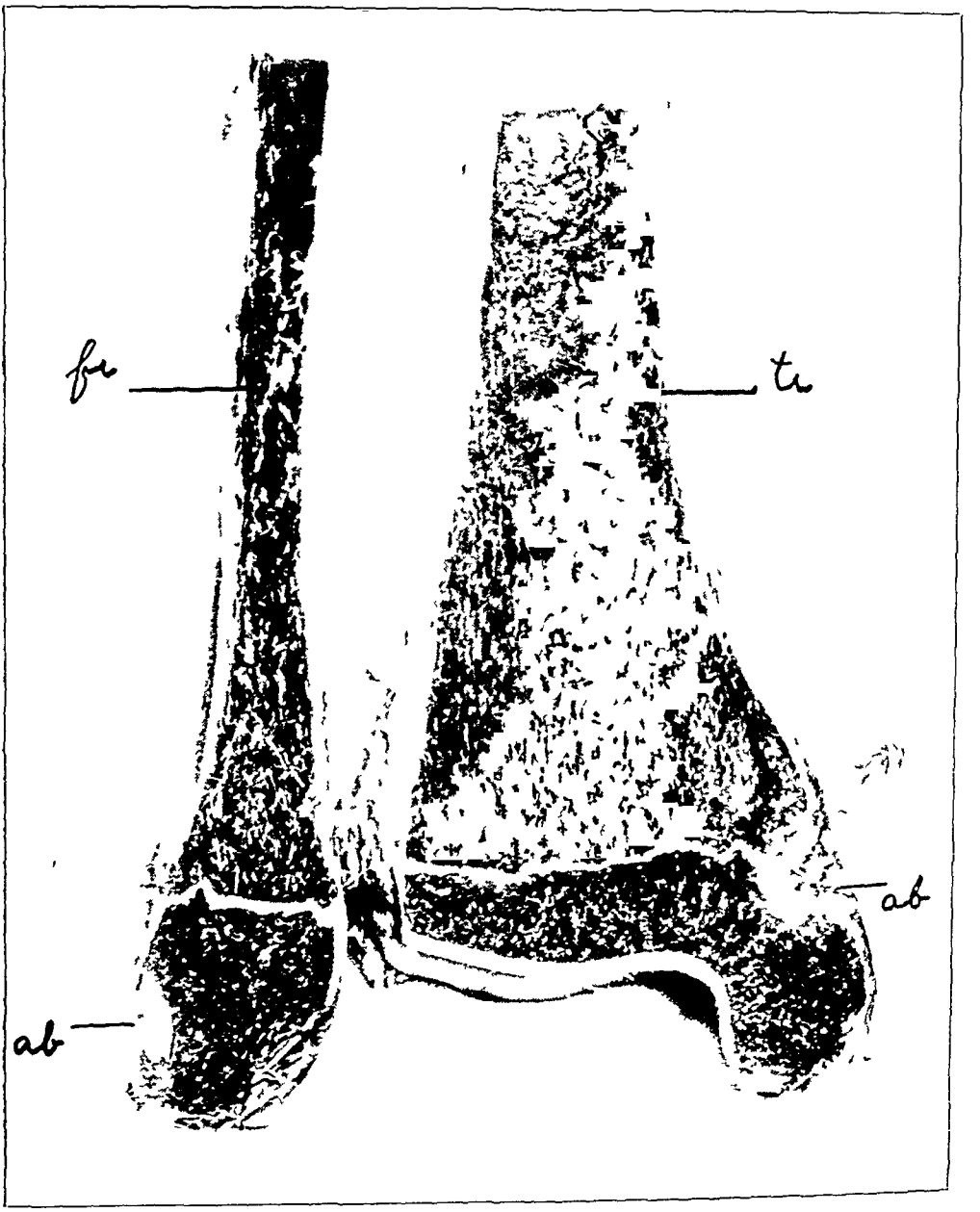


Fig 5 —Longitudinal section through tibia (*ti*) and fibula (*fi*) with abscesses (*ab*) in the epiphyses

Histology—In sections of the indurated region in the left lung, compressed and distorted bronchi were found surrounded by masses of adventitious fibrous tissue one of these bronchi contained enormous numbers of blastomycetes Several of the minute so called abscesses with organisms and the other features of such blastomycotic local lesions were scattered about in this fibrous tissue The

other alterations were those commonly encountered about regions of chronic disease in the lungs

The changes in the bones were of special interest on account of the extensive necrosis Sections were studied from the abscesses in the patella, fibula, femur and tibia, and the alterations were quite similar The centers of small abscesses and the tissue near the surfaces of such as had discharged their contents were made up of disorganized and necrotic bone leukocytes and red cells In the sections from the yellow and soft cancellous bone beneath the articular cartilages



Fig 6—Ulcerations communicating with blastomycotic abscesses in the metatarsus (*mc*) and external malleolus (*em*) Muscles, bones and tendons have been exposed

there was a diffuse necrosis for the depth indicated in the gross description, a necrosis evidently due to the rather regular extension into the head of the bone of the blastomycosis of the joint This diffuse necrosis was difficult to explain by the coalescence of local lesions In some sections zones of mononuclear leukocytes surrounded the necrotic tissue, but no evidences of granulation tissue about

these was found, and organisms occurred outside the lesions in the cavities of the marrow where but little change had taken place Histological changes in accord with the gross description, both indicating the formation of new bone from the cambium, were found in the shell like but perforated and imperfect outer wall of the abscess on the inner surface of the upper part of the tibia

In the inguinal lymph nodes not only were focal lesions present such as have been repeatedly described in the tracheobronchial lymph nodes, but also peripheral lymph sinuses were found crowded with organisms, evidently in the current since the adjacent tissues were without alterations In the spleen, too, organisms were found, at one point a clump of fifteen or twenty, at another, budding forms and about them no evidences of tissue reaction The lesion of the pons, 3 mm from the ventral surface and approximately 1 5 mm in diameter, contained numerous organisms and was apparently formed by the coalescence of more minute local lesions The changes in sections of other tissues and organs conformed to the anatomic diagnosis

Bacteriology—The *Staphylococcus albus* and *aureus* were recovered from the blood, pleural fluid, bile and pus from the abscesses of the knee joints, left inguinal and right axillary regions Blastomycetes were demonstrated in the pus of the abscesses mentioned as well as in the necrotic material from numerous bone lesions, but were not obtained in culture The failure to cultivate these organisms from material yielding cultures during life is not an infrequent post mortem occurrence

This report is of more than ordinary interest on account of the improvement which occurred during the first two periods in the hospital, and also because of the existence of relatively slight blastomycosis in the lungs at the time of death — the organisms having disappeared from the sputum some time previously and coincidentally with the fewer evidences of pulmonary alteration on physical examination — and the very extensive involvement of the subcutaneous tissues, bones and joints

A CASE OF SYSTEMIC BLASTOMYCOSIS

H J MYERS, M D, AND A M SIOBER, M D
CHICAGO

CASE XXII (4).—*History*.—The patient C J S, entered the Cook County Hospital May 8 1907, to the service of Dr Ryerson He complained at the time of dyspnea pain in the right side of the chest, occasional night sweats chills and fever His right ankle was painful and swollen and on the face and left side of the abdomen were cutaneous lesions which his physician had diagnosed and treated as lupus The first of these to occur was a small papule over the eleventh rib on the left side discovered accidentally in December 1906 while scratching his body This ulcerated and gradually increased in size until March 1907 at which time it measured an inch in diameter and was treated as lupus without benefit A similar lesion developed on the left side of the chin after the top of a small papule was cut off during shaving A short time later another lesion occurred over the left eye following the scratching of what the patient thought was an insect bite In January 1907 he first noticed night sweats and several months later a cough associated with bloody expectorations In February he went to Arkansas and while there suffered from chills and fever, which were relieved by quinin At this time he also noticed a tenderness of the right external malleolus which later became painful and swollen together with an abscess of the heel causing a gnawing pain at night and some soreness when the limb was first used in the morning

The patient had been a clerk for three years His previous illnesses were unimportant His father died of gastric carcinoma at the age of 75 From May to November 1906 he was engaged in dredging during which time he slept in a cabin boat which he stated was quite damp at times During June and July he slept with a man who was suffering from what he thought was consumption

Examination.—At the time of admission the patient was poorly developed considerably emaciated and very anemic Crusted lesions were present above the left eye on the left side of the chin and on the left side of the abdomen and there were soft fluctuating abscesses on the left side of the neck and over the right external malleolus, the latter quite red and very painful on pressure Examination of the lungs demonstrated dulness on percussion over the right upper lobe, increased transmission of whispered and spoken voice sounds and numerous small mucous râles No dulness was obtained over the remainder of the right lung or over the left lung Expiration however was prolonged, and the breath sounds somewhat harsh in character A few moist râles were heard over both bases The heart action was strong and forcible, with accentuation of the aortic and pulmonary second sounds The liver extended one finger's breadth below the costal arch the spleen was not palpable The urine was normal, save for an occasional hyaline cast The temperature on the day after admission was 100 F, pulse 76, respirations 22 On May 10 Dr Ryerson made an incision into the swelling over the right external malleolus and considerable pus was evacuated Extending through the periosteum to the epiphysis of the fibula a small sinus was found which from its appearance was considered tuberculous It was enlarged, the cavity curetted swabbed with tincture of iodin, and closed with a small drain The abscess in the neck was also curetted and the region of supposed lupus on the side excised The wounds closed by first intention, but infection in the external malleolus appeared several months later On May 14 four days after the operation, a swelling appeared on the left side of the face and profuse night sweats occurred Pain was felt in the right foot, especially at night,

and a week later the patient experienced sharp, severe pain over the lower part of right chest, this was increased by inspiration and continued for four days The next few days he complained bitterly of pain in both ankles and the right wrist, and Bier's passive hyperemia treatment was tried for some time without, however, producing much benefit On June 7, a fluctuating swelling appeared in the right forearm, and the patient complained of pain in the back between the shoulders

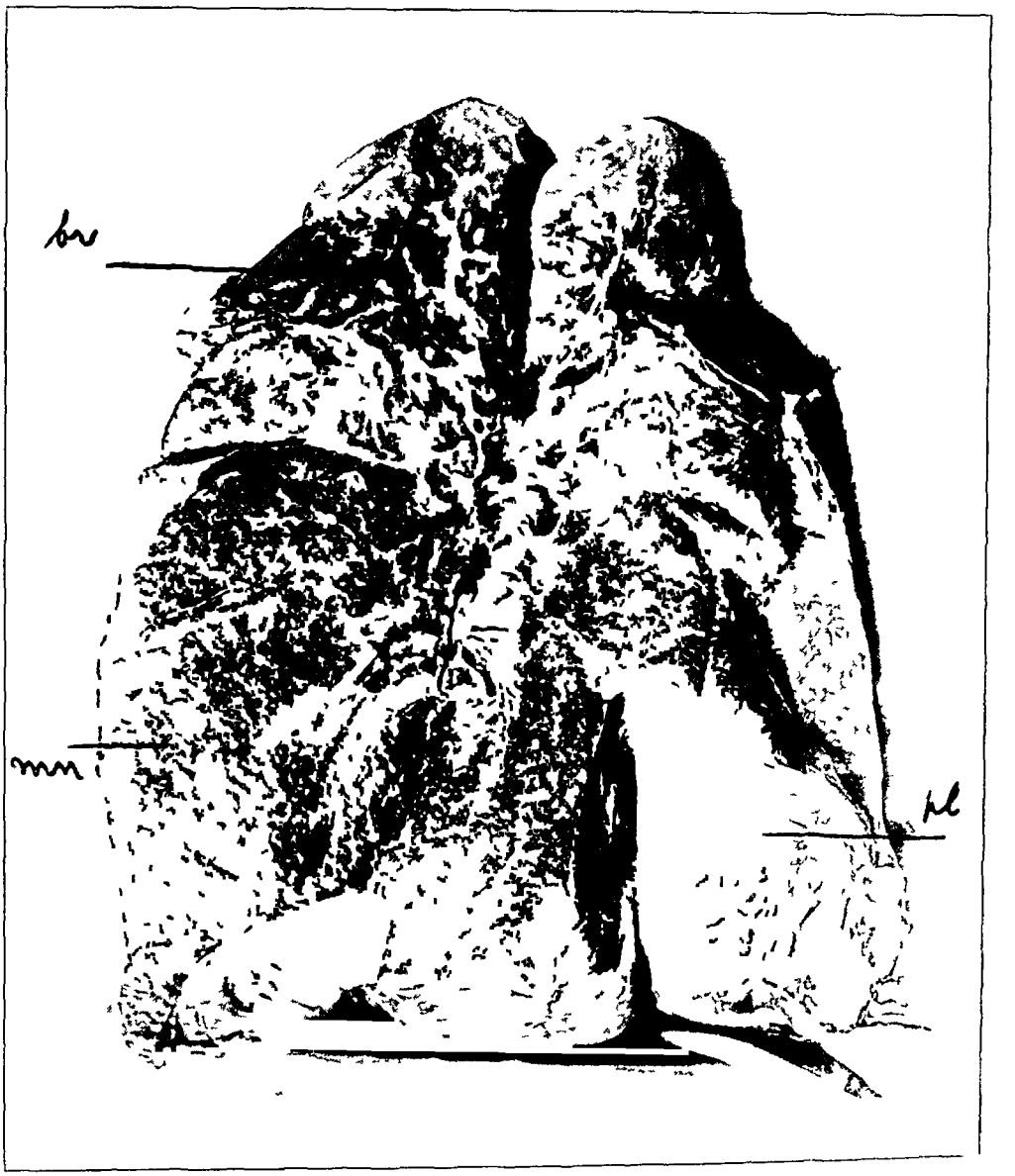


Fig 1.—Right lung, Case 22, *br*, bronchiectatic cavity in upper lobe which has undergone fibrosis, *mn* miliary blastomycotic nodules in lower lobe, *pl*, thickened pleura in which nodules were also present

Treatment and Course.—Up to this time the disease had been considered to be generalized tuberculosis Iron, quinin and strychnin had been administered as tonics, and other remedies as indicated for the pain and insomnia Because a viscid bloody pus obtained on June 11 from an abscess over the left tibia possessed the characteristics of pus from the blastomycotic abscesses in other cases it was

examined with the result that numerous blastomycetes were found Subsequently these organisms were cultivated in pure culture from various abscesses From this time potassium iodid 60 grains, and mercurial inunctions, 1 dram, were given daily A month later the mercury was discontinued and ¼ gram of copper sulphate was given three times a day During the next two months the disease was marked by progressive weakness emaciation and pain in the affected parts especially in the knee and ankle joints The pain was quite constant and so severe that large doses of codein and later of morphin were required for its relief The temperature ranged quite regularly between 99 F in the morning and 102 in the evening Occasional sweats were noted The pulse varied from 80 to 130 beats per minute the respirations from 20 to 26 Toward the end the patient was quite irritable and at times refused both food and medicine

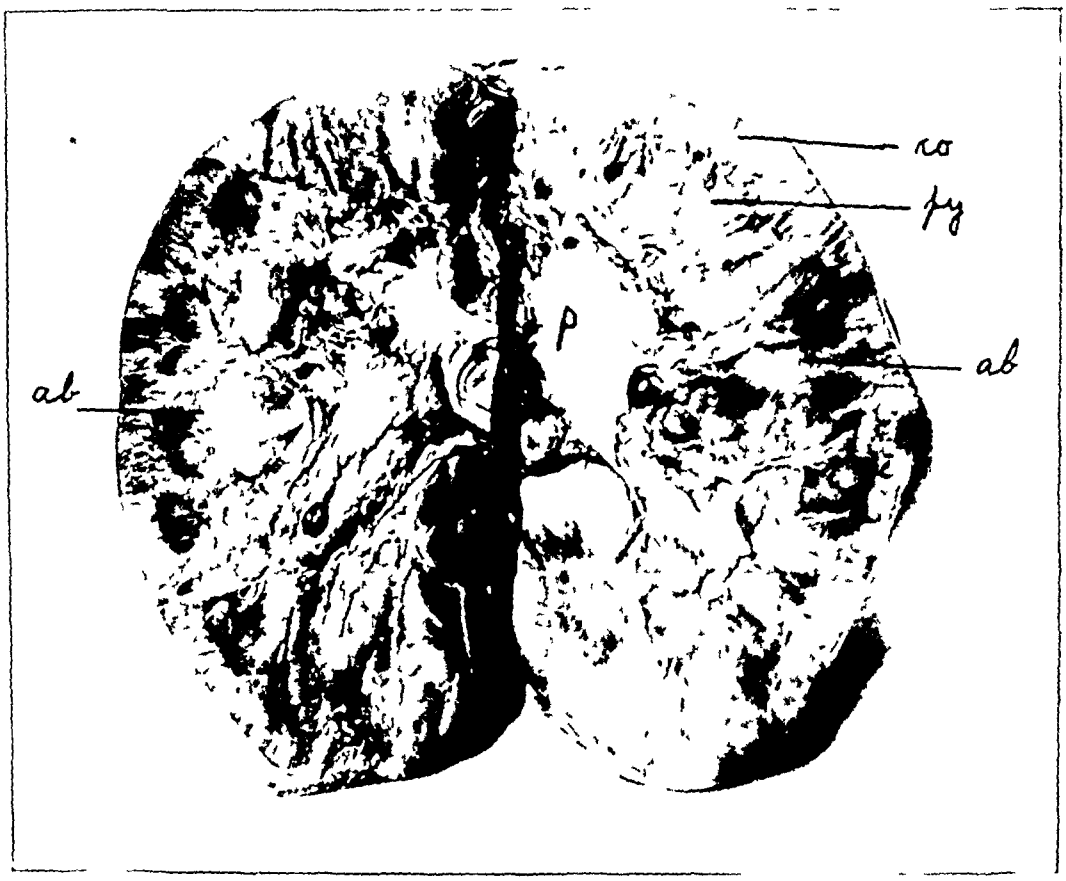


Fig 2—Right kidney, Case 22, *co*, cortex *py*, pyramid, *P*, pelvis, *ab*, blastomycotic abscesses

On September 14, three days before death, five typical ulcers were present on the face Fistulas communicating with abscesses existed over the left mastoid, in both forearms, the left thumb, the inner part of the left thigh, in both knees the tibiae, ankles and dorsal surfaces of the feet Other abscesses were present over the vertex and the occiput, sternum, clavicle, sixth rib, knee, heel, ankle, inner side of thigh and middle of leg—all on the right side, on the left side other abscesses were present over the heel and external aspect of the leg Two large confluent fluctuating regions extended from the seventh cervical to the first lumbar vertebrae Both knees were swollen, fluctuating and tender General adenopathy was present and emaciation extreme The other alterations noted previously had changed but little The patient became delirious a few days before death, which occurred Sept 17 1907

Necropsy—Permission for a post mortem examination was obtained two days after death and the following conditions found Anatomical Diagnosis Healing and discharging blastomycotic abscesses and ulcers of the skin, blastomycotic abscesses of the bones and muscles, blastomycosis of the lungs, peribronchial lymph nodes, liver, spleen, pancreas, kidneys, cerebrum and cerebellum, sero fibrinous and fibrous pleuritis, parenchymatous nephritis, chronic splenitis, hyperplasia of the mesenteric lymph nodes, pigmentation of Peyer's patches, fenestration of the Thebesian and aortic valves, patent foramen ovale, decubital ulcers, emaciation



Fig 3—Lower end of femur which was involved in a suppurative arthritis *ab*, abscess in epiphysis which communicated with the joint, *ca*, eroded articular cartilages

Both pleural cavities were partially obliterated by fibrous adhesions and contained a considerable amount of slightly turbid fluid posteriorly The pleural layers enclosing this fluid were covered by a thin deposit of fibrin Beneath the pleurae of the lungs were a large number of miliary nodules feeling like shot The left lung weighed 900 gm did not collapse, crepitated throughout, and small nodules were felt in its substance The pleura of the lower lobe possessed numerous fibrous and fibrinous tags On section the lung was brownish red and on pressure exuded a large amount of frothy red fluid Many miliary nodules were disseminated throughout the lung At the apex beneath the pleura (torn

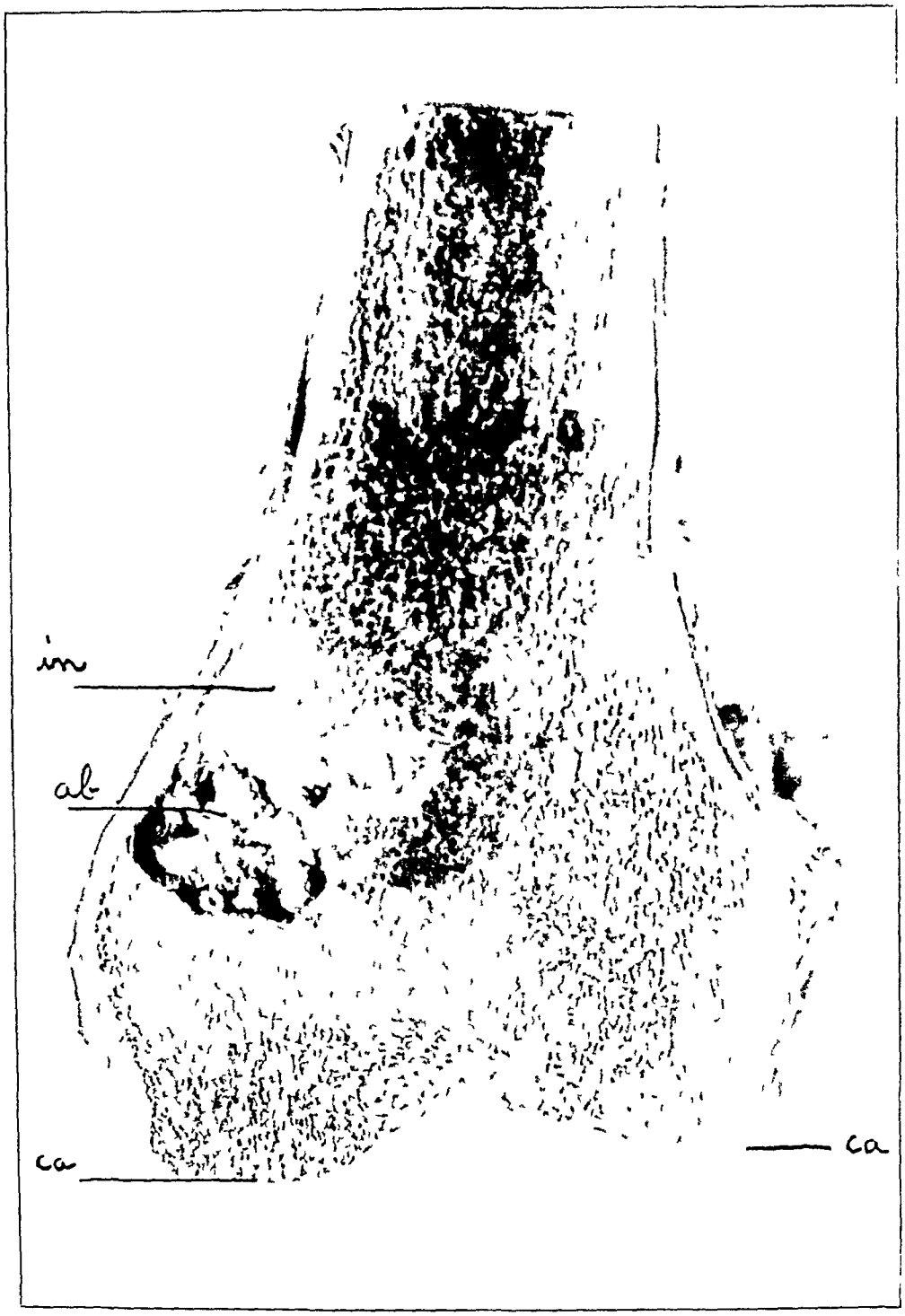


Fig 4.—Longitudinal section of femur shown in Figure 3 *ab*, abscess in the epiphysis, *in*, infected area, the shape of which suggests infarction, *ca*, eroded articular cartilages

in removal) were a number of small nodes from 1 to 1.5 cm in diameter, exuding a purulent fluid on pressure. The right lung weighed 800 gm. The upper lobe was small, firm and covered with a thick fibrous pleura almost cartilaginous in consistence. It contained many solid yellow regions enclosed by radiating bands of fibrous tissue, from which a light yellow purulent material was easily expressed. In the main bronchus of this lung a small opening was found leading into a fistulous passage which extended for a distance of 6 cm. and terminated in a small cavity in the upper lobe. The cavity was irregular, about an inch in diameter and communicated with a small bronchus. Examination of the middle and lower lobes revealed conditions similar to those in the opposite lung (Fig. 1).

The pericardium was normal. In the heart were found a patent foramen ovale and fenestrations of the Thebesian and aortic valves, the other valves were

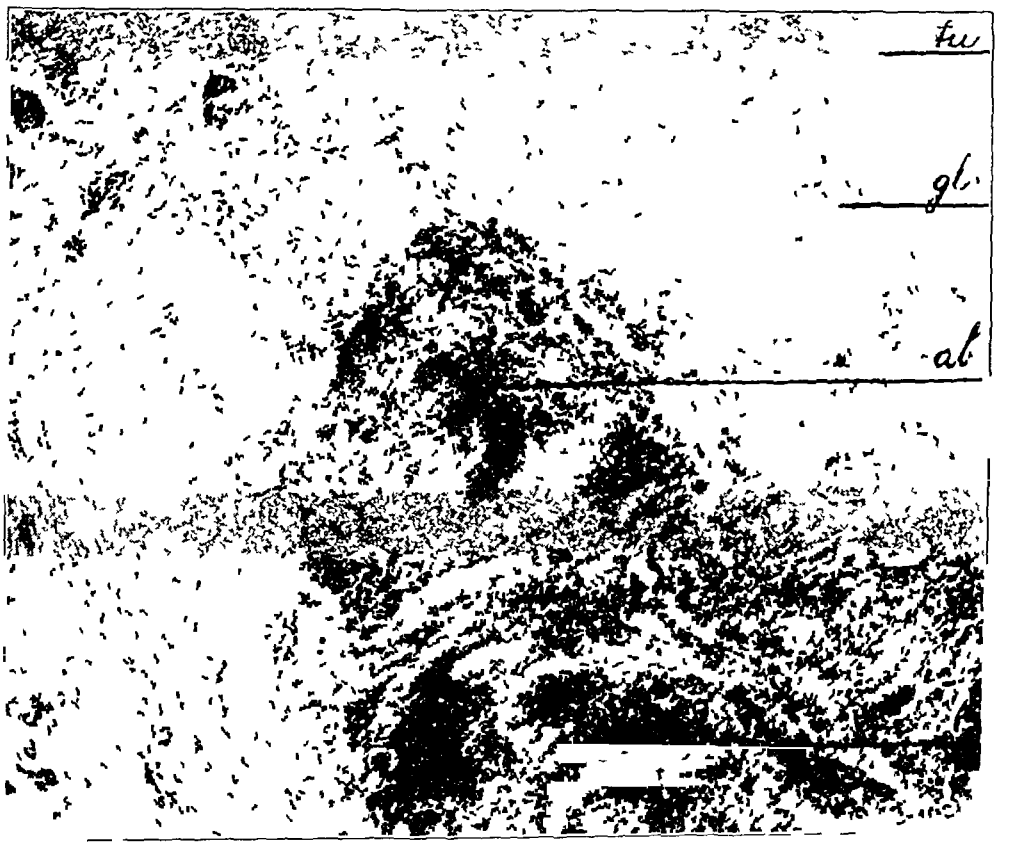


Fig. 5.—Photomicrograph of a blastomycotic abscess of the kidney. *tu*, kidney tubules, *gl*, glomerulus, *ab*, small abscesses which have become confluent.

normal. The myocardium was pale, but no nodules were found in a careful examination.

The pharynx, tonsils, larynx, trachea, and thyroid gland presented no important changes. The anterior portion of the tongue on the left side was somewhat swollen and its surface was roughly granular. The peribronchial and aortic lymph nodes were enlarged and pigmented and contained a number of yellow nodules.

The peritoneum was smooth and shiny. The mesenteric lymph nodes were enlarged but contained no nodules. The liver (1,380 gm.) was smooth and dark red. The cut surface was mottled and after hardening, three very small nodules were found in the interior after considerable search. The spleen weighed 170 gm., was quite firm and had a thickened capsule and trabeculae. Eight small blastomy-

cotic nodules were found in its substance There was a slight atrophy of the idrenals and pancreas and in the latter a small blastomycotic nodule was found The gall bladder and bile ducts were normal The stomach was small, its walls were thickened and the mucous membrane dark gray and covered with tenacious mucus No changes were noted in the intestines, except pigmentation of Peyer's patches

The kidneys were enlarged and except for a depressed scar on the surface of the right kidney the same description applies to both On section the cortex was increased in thickness pale and rolled out beneath the capsule The latter stripped readily leaving a smooth surface and beneath it, as well as on the cut surface were a number of blastomycotic nodules from 2 to 9 mm in diameter

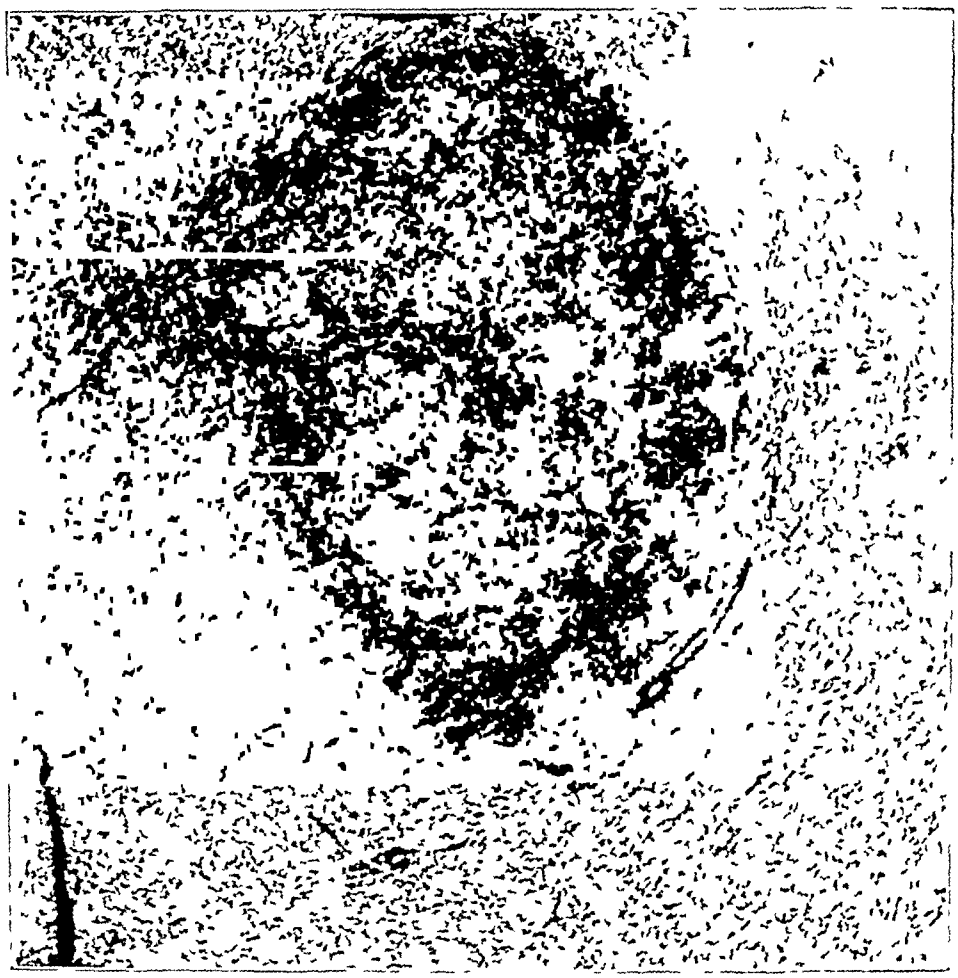


Fig 6—A photomicrograph of a blastomycotic abscess in the cerebrum *cc*, cerebral cortex *cf*, confluent foci of infection

A yellow purulent material could be expressed from the largest of these (Fig 2) The nodules were less numerous in the left kidney The bladder, prostate gland and testicles appeared normal

The brain externally presented no changes After hardening three small blastomycotic nodules were found in the cerebrum and one in the cerebellum

The abscesses beneath the scalp were associated with two deep erosions, one in the occipital bone extending to the dura mater the other in the parietal bone extending to the internal table The abscess over the sternum communicated with an opening in the manubrium On section the greater part of the marrow of the bone appeared to be involved in a suppurative osteomyelitis On the right side necrosis and softening had taken place and an irregular abscess cavity was

present The adjacent marrow compartments were filled with yellow pus to the extent that only a small portion of the left half possessed the normal red color Local abscess formation existed in the first, fifth, sixth and ninth ribs on the right side and in the seventh and eighth ribs on the left side In nearly all cases the abscess involved the entire shaft, producing a swelling an inch or more in diameter, and in some a diffuse yellow purulent infiltration was found extending some distance from the abscesses Another large abscess in the pelvis was associated with an area of bone destruction 10 cm in diameter involving the sacrum and adjacent portion of the right ilium and extending nearly through the bone The right knee presented a diffuse purulent infiltration of the synovial and periarticular tissues, and some erosion of the head of the tibia was found Similar conditions were present about the left knee, which contained a still larger amount of pus The articular cartilages were in some places completely eroded and the surface of the bone hard and smooth (Fig 3) Two communicating openings

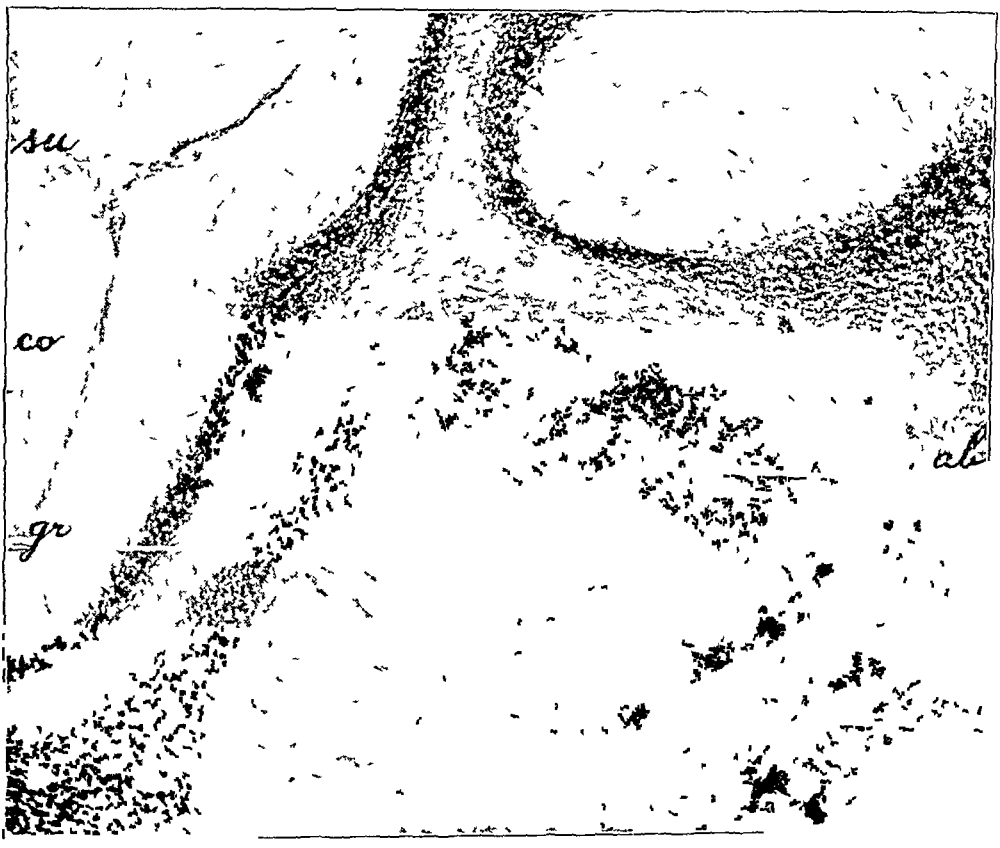


Fig 7 —Photomicrograph of a blastomycotic abscess of the cerebellum *ab*, abscess, *gc*, giant cells, *su*, sulci between the convolutions, *co*, cortex, *gr*, granular layer

into the end of the femur were found, one on the inner side above the internal condyle, and another posteriorly between the condyles In longitudinal section an abscess 2 cm in diameter was found beneath the periosteum, just above the internal condyle The surrounding cancellous bone was softened for a distance of several millimeters Beyond this the bone presented a yellowish appearance in marked contrast to the red color of the unaffected bone The softened area with the abscess was distinctly wedge shape and suggested infarction (Fig 4)

Another lesion involving the periosteum and underlying bone was found in the right tibia at about the middle of the shaft The abscesses about the small bones of both feet and the right wrist were opened and such marked destruction

found that the anatomical structures were recognized with difficulty Blastomycetes were easily demonstrable in the pus of all the bone lesions

Histological examination of the nodules found in the various viscera demonstrated the blastomycotic nature of all of them They possessed so many features in common that it is not essential to describe them in detail (Fig 5) A rather marked peculiarity was found in the sections of the single nodule of the pancreas, for without the connective tissue wall surrounding a region of necrosis and numerous organisms there was no enveloping zone of round cells as is usually found

The nodules in the cerebrum appeared as though they were caused by the localization of a considerable number of organisms at adjacent points (Fig 6) The location of the nodules in the cerebellum were chiefly in the granular layer, and an apparent connective tissue wall was present in and about some of them (Fig 7)

In the lesions of the bone well marked walls of granulation tissue were not present about the necrotic areas and in the adjacent marrow compartments organisms were found unassociated with any tissue reaction

Bacteriology—Cultures were made at the necropsy from the blood pleural fluid and the pus from various abscesses which had not been opened during life No growth was obtained from the blood or the pleural fluid, but from the pus pure cultures of blastomycetes were obtained They differed in no respects from those obtained during life from the pus of other abscesses and a report of their cultural characteristics will be included in a subsequent comparative study

Thanks are due Dr E C Rverson attending surgeon for permission to report this case

A CASE OF BLASTOMYCOSIS

T H BOUGHTON, M D, AND S N CLARK, M D
LAWRENCE, KAN HOSPITAL, ILL

CASE XXIII (5) —*History*—J C, man, aged 21, entered Cook County Hospital, Aug 21, 1907, to the orthopedic service of Dr E W Ryerson He was born in Italy, moved to the United States at the age of 1 year, and lived in Chicago since He went to work at the age of 14, and has been engaged in peddling and factory work For several months previous to his illness he worked in a tobacco factory and inhaled considerable tobacco dust He also chewed considerable tobacco while in the factory When at home he lived under fair hygienic conditions, but during the fall preceding the onset of the disease, after getting into trouble and being expelled from home, he slept in exposed out buildings and basements The previous and family history are unimportant

He caught cold Nov 15, 1906, and was confined to the house for two weeks, coughing frequently and expectorating bloody sputum at this time He went to work December 1, and noticed pain in back of neck, and in right side of chest, and was obliged to stop work in a few days because of weakness especially in the left arm, and was then confined to bed until December 27 At this time he suffered with pain in the back, bloody expectoration and night sweats After January 1 he improved sufficiently to go to work, but, owing to weakness, was again obliged to return to his home, where he remained for the next five months He coughed, expectorated bloody sputum, suffered pain in back and was quite weak On May 30 he fell and hurt his back From that time urination and defecation were involuntary Two days after the fall he came to the hospital, complaining of pain in side, which was strapped, and he returned home, but the pain persisted for two weeks On July 1 he was sent to Dunning, where he remained for one month in the tuberculosis hospital, two weeks of this time he was in bed On August 21, as previously stated, he entered the hospital complaining of painful cough, weakness of the legs and involuntary evacuations A kyphosis at the level of the second dorsal vertebra was noticed at this time and in the examining room the disease was considered tuberculosis of the lungs and spine

Three weeks after entering the hospital (September 15), he fell while walking about the ward, and was thereafter unable to walk, owing to partial paralysis and about one month later the paralysis of the legs became complete Sensation was much diminished in the legs, but was never completely lost The knee jerks were increased, ankle clonus and Babinski's sign also were present Up to this time the diagnosis of tuberculosis had not been questioned, but the failure to find the tubercle bacilli in the sputum, together with the presence of numerous cutaneous lesions, gave rise to suspicions of blastomycosis Examination of the unstained sputum and pus from various abscesses was undertaken with the result, at this time quite surprising to us, that the organisms were found quite abundantly present in both

Examination—An examination about December 1 revealed the following conditions The patient lies quietly, seems comfortable, is unable to move the lower, and experiences some pain on motion of the upper extremities Emaciation is marked and the skin is harsh and dry He coughs occasionally and expectorates a purulent blood tinged sputum The voice is husky and evacuations are involuntary On the left cheek near the nose is a dull red, slightly indurated, sharply outlined, flat crusted area 2 5 cm in diameter, marking the rupture of an abscess, part of which remains as a slightly red fluctuating mass about 3 cm in length,

extending downward and outward On the right cheek in front of the ear, is a reddish pigmented scar 2 by 3 cm at the upper portion of which is a sinus communicating with an abscess the size of a hickory-nut There is an abscess several times larger at the base of the neck on the left side The cervical lymph nodes are enlarged Several small firm painless irregularities are present on the ribs, and a tender point on the right fourth rib in the axillary region The lungs are hyperresonant with the exception of the right apex interiorly and the upper lobes and upper portions of the lower lobes posteriorly over which resonance is impaired and dry rales bronchial breathing and bronchophony are heard Over the remainder of the lung the respiratory sounds are roughened and prolonged The area of heart dulness is normal the tones clear and regular

The abdomen is distended and tympanitic and contains no masses or tender regions The liver extends to two fingers below the costal margin The spleen is easily palpable and on deep inspiration extends three fingers' breadth below the costal arch At the point of the kyphosis previously mentioned a large flat abscess is present which is 10 cm in diameter and on the posterior surface of the right shoulder is another, soft and fluctuating and 7 cm in diameter A third is present on the left arm and from it small quantities of bloody pus escape Decubital ulcers are present on both heels and over the sacrum

An examination of the nervous system demonstrated no changes in reflexes of eyes and upper extremities The abdominal and cremasteric reflexes are present although less marked on the left side The lower extremities are paralyzed and foot drop is marked The patellar reflexes are very weak and the Kernig and Babinski reactions, the Achilles reflex and the ankle clonus are all now absent The plantar reflexes are exaggerated and stimulation of the plantar surfaces causes flexion of the thigh and leg although voluntary motion is absent Sensation to heat and cold are entirely lost and to touch and pain much diminished

Leukocytes and granular casts were often found in the urine which was usually of low specific gravity Albumin was absent until the last part of his illness and no blastomycetes were found in the sediment

An examination of blood Dec 5 1907 showed red cells 4 972 000 hemoglobin 75 per cent (Tallqvist) leukocytes 20 800 In a differential count there were neutrophils 84 3 per cent eosinophils 0 basophils 0 small lymphocytes 7 3 per cent, large lymphocytes 8 3 per cent Enormous numbers of blastomycetes were regularly found in the sputum but no tubercle bacilli were demonstrated in repeated examinations Bouillon inoculated with blood removed from the midvein of the arm Dec 3 1907 remained sterile Subcutaneous inoculation with tuberculin Nov 14 1907 produced no reaction, but a conjunctival reaction was obtained ten weeks later

The patient remained in the hospital until his death which occurred March 14, 1908 a residence of seven months The course was progressively downward, though some of the symptoms were much abated and there were periods when the general condition seemed much improved During November the cough which at first was very troublesome and painful ceased to be annoying The sputum, which in the beginning was profuse and blood tinged became much less in amount and the blood disappeared During January a small amount of voluntary motion returned to the legs The abscess over the lumbar spine disappeared entirely, but the abscess at the base of the neck became larger and the kyphosis more pronounced, and some crepitus could be obtained over this region About January 1, pain and tenderness appeared in the left knee and soon afterward both knees and ankles were involved in a suppurative process, which formed discharging sinuses These joint manifestations were extremely painful, especially during the last month of life

The temperature was about normal most of the time, often it was subnormal, and occasionally it rose as high as 101 F for a few hours on a day The pulse was rather rapid but extremely variable, ranging from 70 to 130 per minute The

respirations ranged from 22 to 28 During the last two months of life the pulse was constantly above 100, the temperature usually normal or subnormal (occasionally as high as 100) and the respirations averaged about 26

The patient was given tonics until December 1, calcium iodid, 2 grains, every half hour until January 18, and then potassium iodid from 10 to 30 grains, four times a day

Necropsy.—(Dr Stober)—Permission for a limited necropsy was secured March 15, one day after death, and performed under circumstances which are responsible for the incompleteness of certain portions of the examination The body had been embalmed and cultural work was not possible

Anatomical Diagnosis Multiple blastomycotic lesions of the skin, subcutaneous tissues, muscles and bones, blastomycosis of the lungs, peribronchial lymph nodes, and spleen, passive congestion and edema of the lungs, parenchymatous degeneration of the kidneys and liver, bilateral fibrous pleuritis, decubital ulcers, anasarca, kyphosis, general emaciation

The upper part of the body showed extreme emaciation in contrast to the abdomen and lower extremities, which were markedly edematous Decubital ulcers the size of the palm were present over both hips On the left side of the face was a circular scar with margins still red and slightly crusted A small fluctuating area could be felt in the deep tissues beneath Another marking the site of an old abscess was present in front of the right ear Behind the right shoulder was found a large fluctuating abscess which seemed to be confined to the subcutaneous tissues, a much larger one was found over the upper part of the spine extending to either side for a distance of several inches A kyphosis of the spine involving the last cervical and the first two dorsal vertebrae was present at this point The spines of the vertebrae were separated from each other more than usual, and manipulation of the spine of the first dorsal, which was the most prominent, elicited a distinct crepitus The kyphosis could be reduced and exaggerated to a considerable extent by extension and flexion of the neck on the chest An external incision was here not allowed

An examination of the fistulas connecting with underlying abscesses over both knees and ankle joints demonstrated eroded bone at their bases, but further examination of the bones was not allowed On opening the chest, the pleural cavities were found practically obliterated by fibrous adhesions These were very firm at the apices, especially on the right side, where dense cicatrical masses were present The lung was torn at this point on removal and presented a fistulous opening which communicated with a cavity in the upper lobe, and extended posteriorly between the first and second ribs, the adjacent margins of which were sharp and eroded No direct communication with the abscess in the back could be demonstrated by pressure on the latter

The bodies of the vertebrae from the front appeared normal The anterior surface of the second thoracic was covered with the fibrous tissue involving the right pleura This vertebra was removed with the bone forceps, and a small portion of the spinal cord was exposed but appeared normal The cancellous tissue of the bone was normal Further examination of the vertebrae and back was not allowed

The lungs together weighed 1 100 gm Both showed thickened pleurae with numerous fibrous tags The upper lobe of the right lung was torn in removal as described This lobe was small and quite solid, although slight crepitation could be obtained in the lower portion In the upper portion of the lower lobe and in the middle lobe could be felt a number of nodules On section, the greater part of the posterior portion of the upper lobe was occupied by an irregular cavity which communicated with one of the bronchi The main bronchus was dilated to form another small cavity at about an inch from the bifurcation The surrounding tissue was fibrous in character and contained a number of yellow necrotic regions measuring from 3 to 6 mm in diameter Between these and also in the anterior portion of this lobe, the middle lobe and the upper half of the

lower lobe were numerous miliary nodules, some of the larger of which were necrotic Coal pigment was present to a considerable extent especially in the affected parts of the lung Considerable frothy fluid could be expressed from the lower lobes (Fig 1) The left lung crepitated freely throughout except at the apex where a large radiating scar was present extending into the lung for a distance of several centimeters On section, the lung was dark red and bloody frothy fluid could be expressed Small nodular fibroid masses throughout the

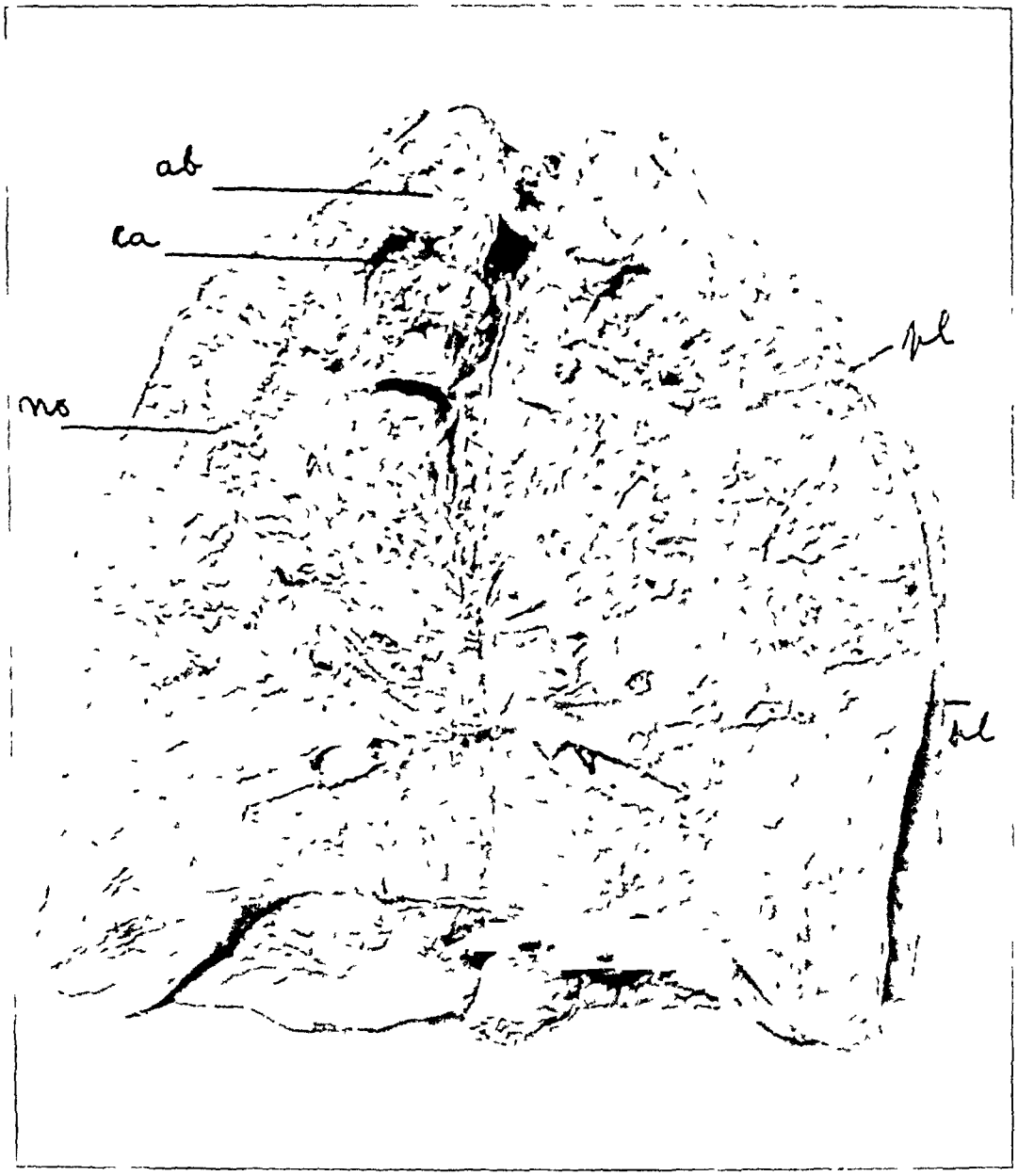


Fig 1—Right lung, Case 23 *ca*, bronchiectatic cavity in the fibroid upper lobe, *ab*, abscesses whose contents were inspissated and surrounded by a wall of connective tissue, *no*, blastomycotic nodules in the lower lobe, which from their distribution seem to be bronchogenic in origin, *pl*, thickened pleura

lung were cut through The bronchi were here as well as in the opposite lung thickened and prominent The peribronchial lymph nodes were enlarged, from 1 to 3 cm in diameter, and dark red to black on section Two of them contained several small encapsulated necrotic regions and also a few miliary nodules

The heart, empty, weighed 255 gm The pericardium on the anterior surface presented an area of fibroid thickening 3 cm in diameter The valves appeared normal The musculature was a light brown, and in numerous sections no nodules were found Nothing of importance was found in the thyroid, esophagus, stomach, intestines, pancreas, bladder, prostate or adrenals The liver was much enlarged and its anterior margin was considerably rounded The cut surface was light yellow and fat droplets could be seen in the material scraped from the surface On section of a portion of the right lobe one small nodule 2 mm in diameter was found The spleen was moderately enlarged The surface was smooth and the capsule somewhat thickened On section an excess of connective tissue was present, the Malpighian bodies were indistinct and a few miliary nodules were found

The kidneys together weighed 260 gm and were normal externally The cortex was increased, measuring 7 mm in thickness, and was pale yellow in contrast to the pyramids, which were light red and measured 20 mm in length The capsule stripped readily, leaving a smooth surface No focal lesions were found in either organ

Histological Examination.—Some difficulty was experienced in demonstrating the organisms in the necrotic material described in the right lung as being surrounded by more or less marked masses of fibrous tissue The lesions in other organs were similar to those described in other cases Their blastomycotic nature was proved with the exception perhaps of the sections examined of the small nodule in the liver, in which no organisms were found In other respects this too corresponded to blastomycosis The sections of the other organs possessed changes indicated in the anatomical diagnosis No lesions peculiar to blastomycosis were found in sections of the body of the second dorsal vertebra

Bacteriology.—Blastomycetes were repeatedly demonstrated in the sputum and in the pus from the cutaneous lesions and the various abscesses during life, and pure cultures were obtained from the pus when the abscesses were aspirated or drained In the mucus removed from the trachea and bronchi at the time of the post mortem examination the organisms were found

A CASE OF SYSTEMIC BLASTOMYCOSIS WITH RECOVERY

T. H. BOUGHTON, M.D. AND V. M. STOBER, M.D.
LAWRENCE, KAN. CHICAGO

CASE XXIV (6).—*History.*—S. K., a native of Montenegro aged 39, was admitted to the service of Dr. Slavmaker April 12, 1908. It was learned through an interpreter that he had been in this country one year, working first as a laborer on a railway for five months, then for two months in Missouri laying pipes. During this time he spent half of each day in a ditch partly filled with water, but declares that he felt perfectly well. The next five months he lived in South Chicago and the last three months he was ill, owing to exposure while looking for work. He lived about two blocks from the lake on the second floor of a frame house built on low ground which was wet and muddy at that time of the year, November, 1907, to March, 1908. Seventeen persons slept in the five rooms on this floor, two or three of them were sick in bed with fever, and although he was unable to give many details of their illness he stated that there was a great deal of coughing. A small cooking stove in a hall was the only source of heat for the entire second floor of this building.

No history of venereal infection was obtained and the family history was unimportant. This illness began in January, 1908, with intense pain in the chest, cough, fever and weakness. Blood was present in the sputum at first and has continued to appear at intervals since. During the two weeks before his admission to the hospital a number of abscesses developed and the dorsal surfaces of both feet became swollen and tender. He has lost some weight but does not know how much. When he entered the hospital he complained of pain in the feet and ankles. An examination at this time revealed a slightly reddened superficial ulcer about the size of a finger nail over the left supra-orbital ridge. It was covered with a thick crust under which was bloody pus. Similar lesions were present on the lower right eyelid near the nose and over the angle of the right scapula, and also small pustules on each arm just above the left external malleolus. There were three smooth pigmented scars on the right thigh which the patient ascribed to some running sores connected with the bone, which he had when a boy.

He was well developed and well nourished and obviously not very sick. There was no evidence of consolidation found in the examination of the lung. Slightly impaired resonance was obtained over the right apex and a few crepitant and subcrepitant râles were heard over the region and over both lower lobes. The area of cardiac dulness was increased to the left 2 cm. The inguinal lymph nodes were moderately enlarged.

About May 1 no râles could be heard in the lungs. Otherwise the pulmonary examination disclosed nothing new. A crusted lesion had appeared on the back of each hand. The pustule on the left leg had developed into an ulcerated crusted lesion 3 cm. in diameter and discharged considerable bloody pus. A subcutaneous abscess 5 cm. in diameter which had developed about the right external malleolus was opened and a large amount of dark bloody necrotic material escaped, and in this blastomycetes were found and pure cultures obtained.

The upper end of the right tibia was very tender, and the overlying skin was red and edematous. The dorsal surfaces of the feet were swollen and tender, walking was decidedly painful and the patient's general condition was somewhat worse. A month later the patient was confined to bed by weakness. The tenderness and redness over the right tibia had disappeared and a small subcutaneous

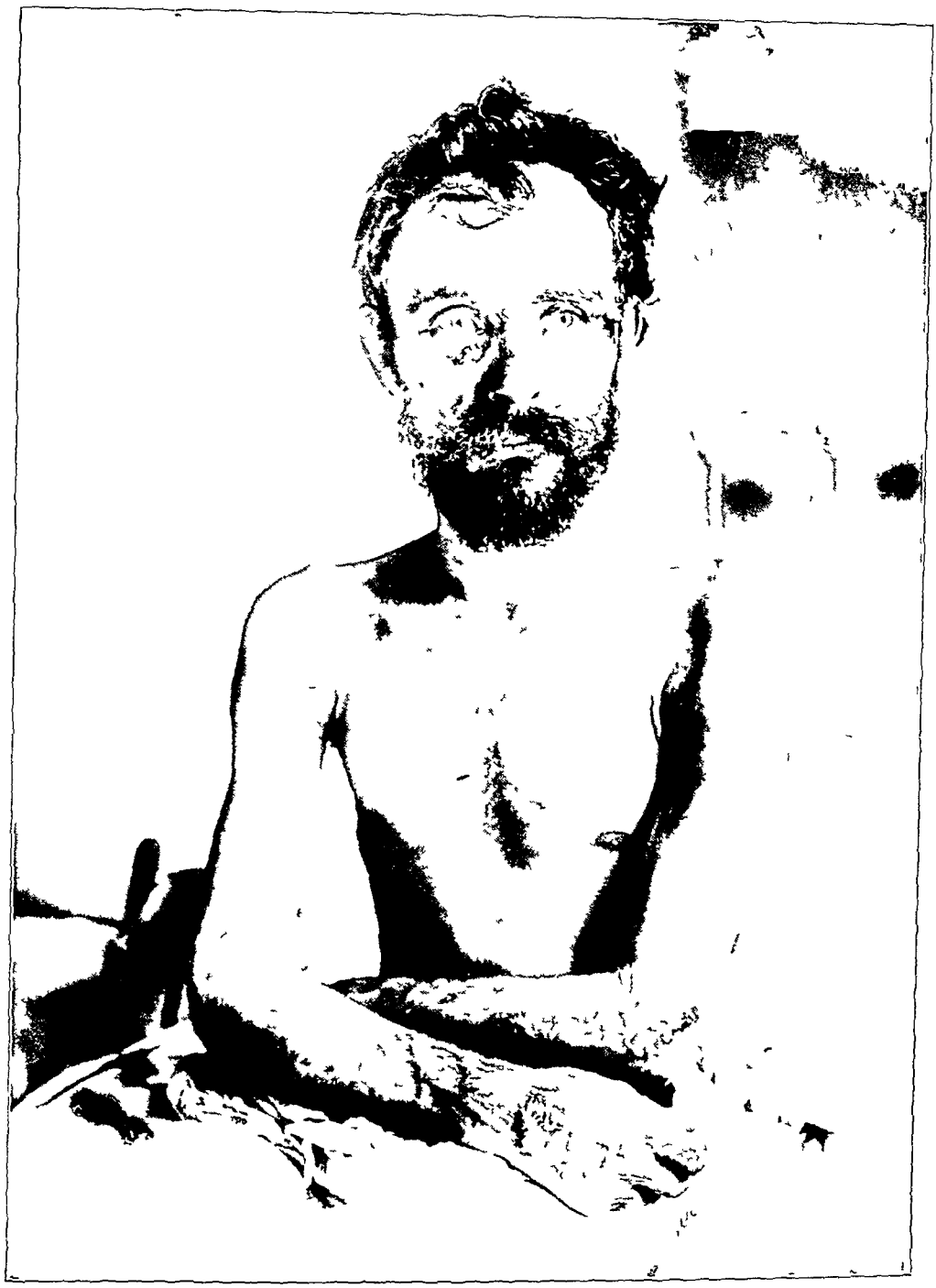


Fig 1.—Case 24. This photograph was taken shortly after the vaccine treatment was begun. Three lesions on the face, three on the left forearm and one on the right hand can be distinctly seen.

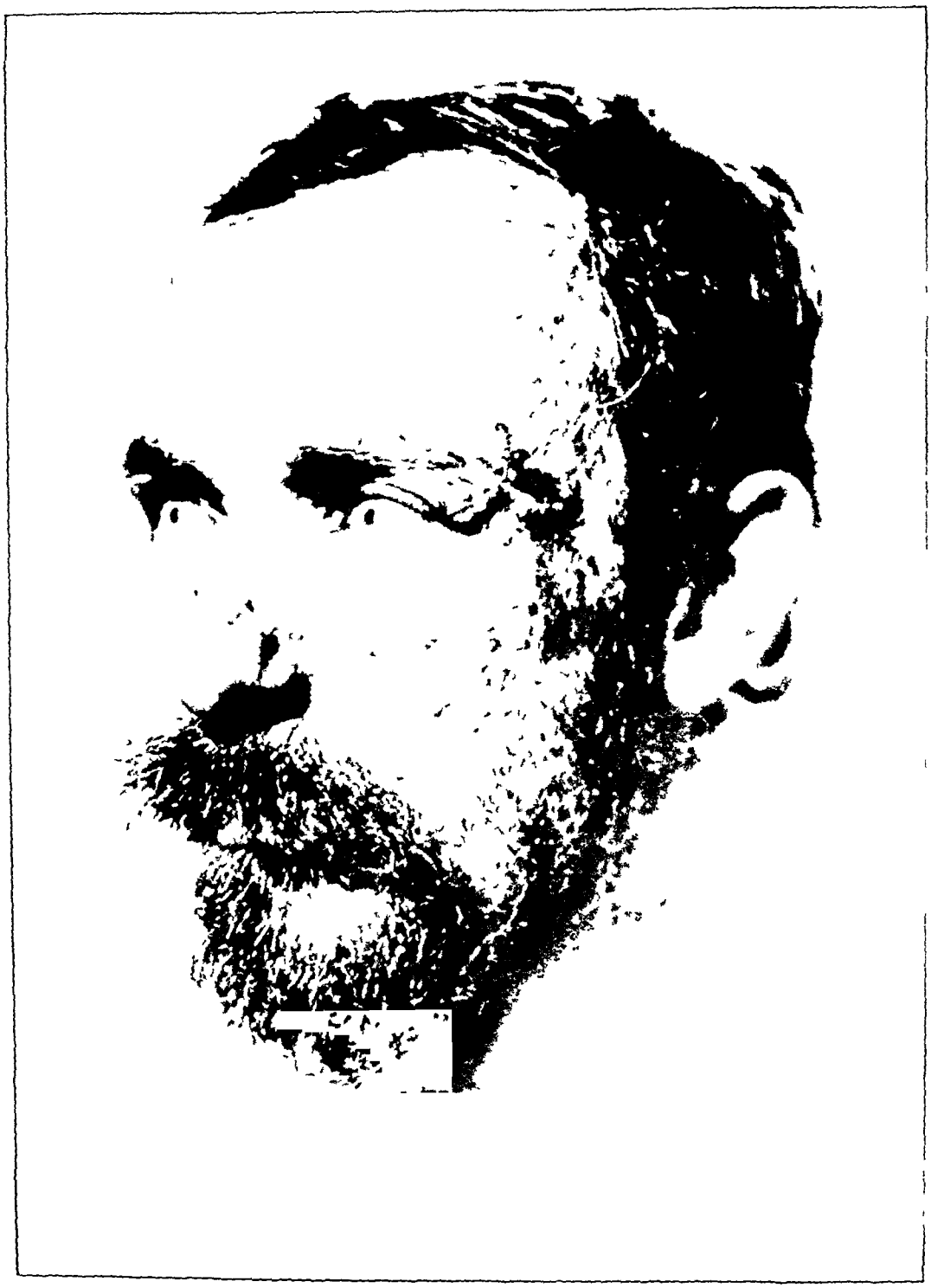


Fig 2—Case 24 Taken during the latter part of August six weeks after beginning of vaccine treatment The lesions on nose and under right eye have disappeared The one present near left eye is much improved

abscess had developed near the attachment of the patellar ligament The tenderness and swelling on the dorsal surface of the right foot had disappeared completely, and a small subcutaneous abscess had developed on the dorsal surface of the left foot A small area of redness, tenderness and swelling had appeared over the middle of the left tibia, and a small pustule had developed over the spine of the right scapula The sputum was still occasionally tinged with blood

During June two small lesions developed on the nose, one on the right cheek (Fig 1), six lesions on the left arm (three on the extensor and two on the flexor surface of the forearm, and one on the back of the elbow), one on the back of the left thigh, one on the anterior surface of the right thigh, and one on the outer aspect of the left leg below the knee making a total of twenty four distinct superficial lesions, only six of which were present on admission

It is interesting to note that the arrangement of the lesions was roughly symmetrical Until July 10 improvement was noted in only two of the lesions in spite of the fact that patient had been under treatment with potassium iodid and copper sulphate from the time of the discovery that the disease was blastomycosis and not rheumatism, for which he had previously been treated One of these lesions was a small cutaneous abscess at the angle of the scapula which had never been large or deep This had ceased to discharge and at this time was red and indurated The other lesion was a swollen tender area on the dorsal surface of the right foot which lasted about three weeks and did not suppurate

The sputum on repeated examinations showed no tubercle bacilli, but constantly a few blastomycetes The urine in a number of analyses showed nothing abnormal An examination of the blood made early in the course, April 30, 1908, demonstrated 5,748,000 red cells and 90 per cent hemoglobin (Talqvist) The white cells numbered 18,300, of which the polymorphonuclear cells constituted 75 per cent, small mononuclears 17 per cent, large mononuclears 6 per cent and eosinophils 2 per cent

Vaccine Treatment.—A vaccine treatment was instituted July 10, and consisted of the subcutaneous injection of the filtrate and suspension of the blastomycetic mycelium obtained from cultures grown in glucose bouillon at room temperature The temperature up to June 1 ranged from 99 to 100 4 F and from June 1 to July 10 from 99 to 99 8 F Seven hours after the first injection (July 10) a maximum temperature of 100 8 F was noted, returning to normal after two days and remaining so except after the subsequent injection of vaccines, when increases of from 0 4 to 1 4 F were obtained With the rise of temperature occurred also a distinct inflammatory reaction about the lesions consisting of pain, redness, swelling and increased discharge, and also a general feeling of malaise After the second injection, July 15, an active healing of the lesions occurred The vaccine used in the third injection, July 22, was prepared from cultures of a mold obtained from wood (Fig 19, p 537), and the reaction obtained was even more marked and prolonged than from the vaccine prepared from the cultures of blastomycetes A deep abscess in the right arm suddenly enlarged, approached the surface and ruptured The site of the injection also became red and indurated and from the swelling a few days later a small quantity of sterile pus was aspirated The patient by this time was able to walk about in the yard

Two more injections were given August 10 and August 24, with the result that on September 1 seventeen of the twenty four lesions previously noted had disappeared The lesions remaining were still red and indurated but the discharge had ceased and they were much smaller (Fig 2) The healed or healing condition of the bone lesions was quite apparent in roentgenograms taken at this time and reference to these is made by Dr Potter, roentgenographer, in a review of the subject (p 541) On examination of the chest impaired resonance was obtained over the right lung posteriorly with harsh breath sounds over this area and the upper portion of the left lower lobe A few dry rales were heard over the right side The patient's general condition was much improved and

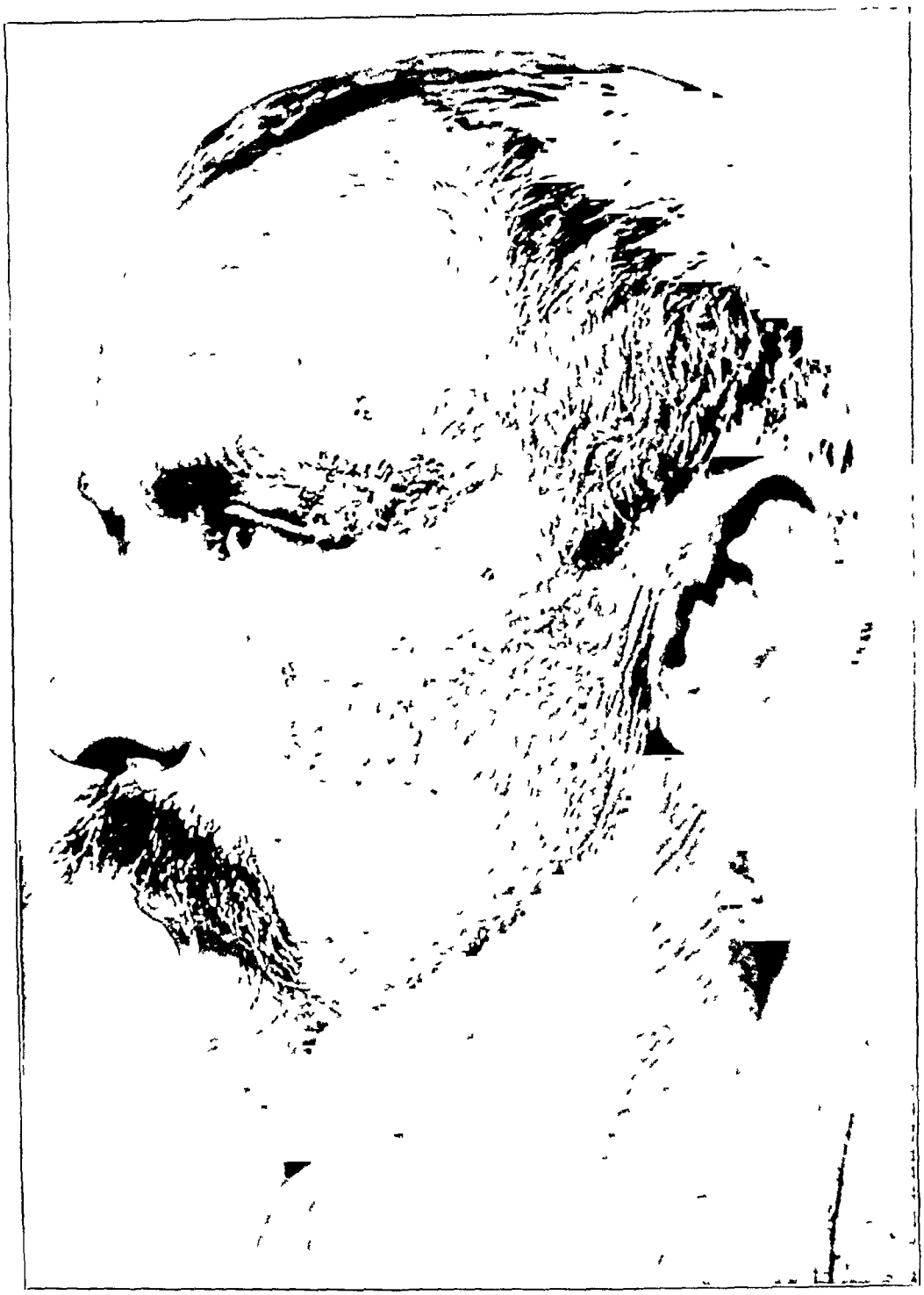


Fig 3—Case 24 Taken during latter part of September, showing nearly healed condition of lesion near the left eye

he gained considerably in weight Other injections were given September 17, October 13, October 31, November 15 and December 1, when they were discontinued Of the twenty seven lesions, all but two seemed to be healed one on the right forearm, and one on the left side of the forehead (Fig 3) The patient had gained much in weight and felt better

The results of the blood examination made September 17 were as follows white cells 11,000, polymorphonuclears 65 6 per cent , small mononuclears 21 8 per cent , large mononuclears 10 per cent , transitional cells 1 8 per cent , eosinophils 0 6 per cent , and myelocytes 0 0 per cent On September 19, 15,300 whites were found, an increase of 4,300 two days after injection of vaccines Other

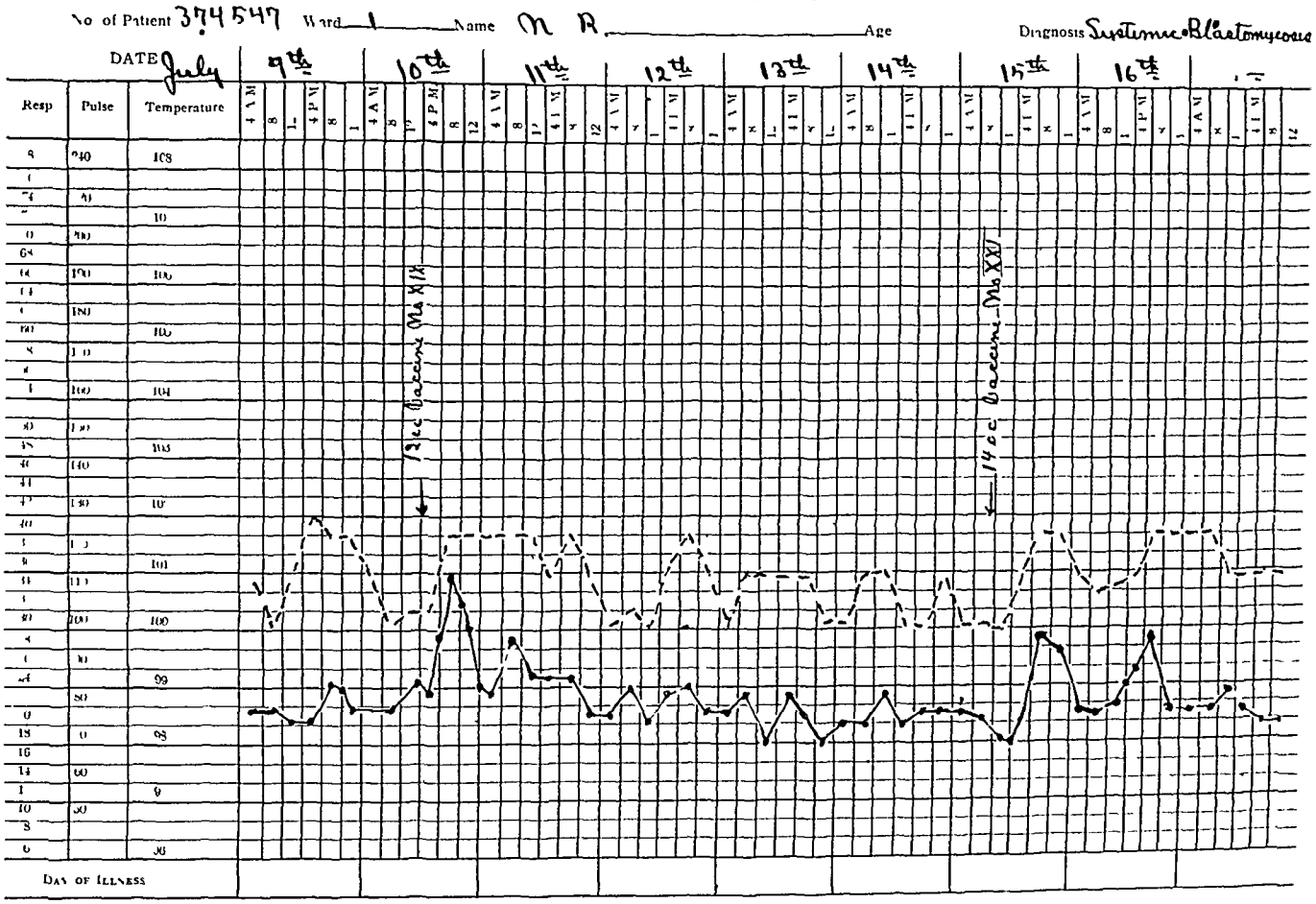


Figure 4

counts October 13 and October 31 showed little variation from the above except for the increase of large mononuclears to 17 per cent and the presence in some of these of fine neutrophilic granules, thus approaching the myelocyte in type

On December 9 the patient furnished the blood for a venous transfusion and was quite weak for a few days but seemed to regain his strength During January he began to lose weight and the lesion on the right forearm began to discharge On February 1 a small papillomatous growth appeared on the right ala of the nose and an abscess on the dorsum of the right foot The latter was opened and drained and the administration of potassium iodid and vaccine (2 c c every tenth day) was again resumed, with the result that by April 1 the lesions

on the arm and nose were practically healed, but the abscess on the foot was still discharging slightly On April 21 he began to complain of a pain in the left chest which increased in severity and was associated with the appearance of physical signs of fluid in the left chest On May 4 the left chest gave a flat note on percussion below the level of the middle of the scapula, voice sounds were diminished and breath-sounds absent At this time the patient was confined to bed and given potassium iodid in doses increased for 20 to 85 grains three times a day and vaccine filtrate from 1 to 3 c c every tenth to fourteenth day A gradual improvement occurred and on June 1 the patient was allowed to walk about in the yard All evidences of fluid in the chest had disappeared He left the

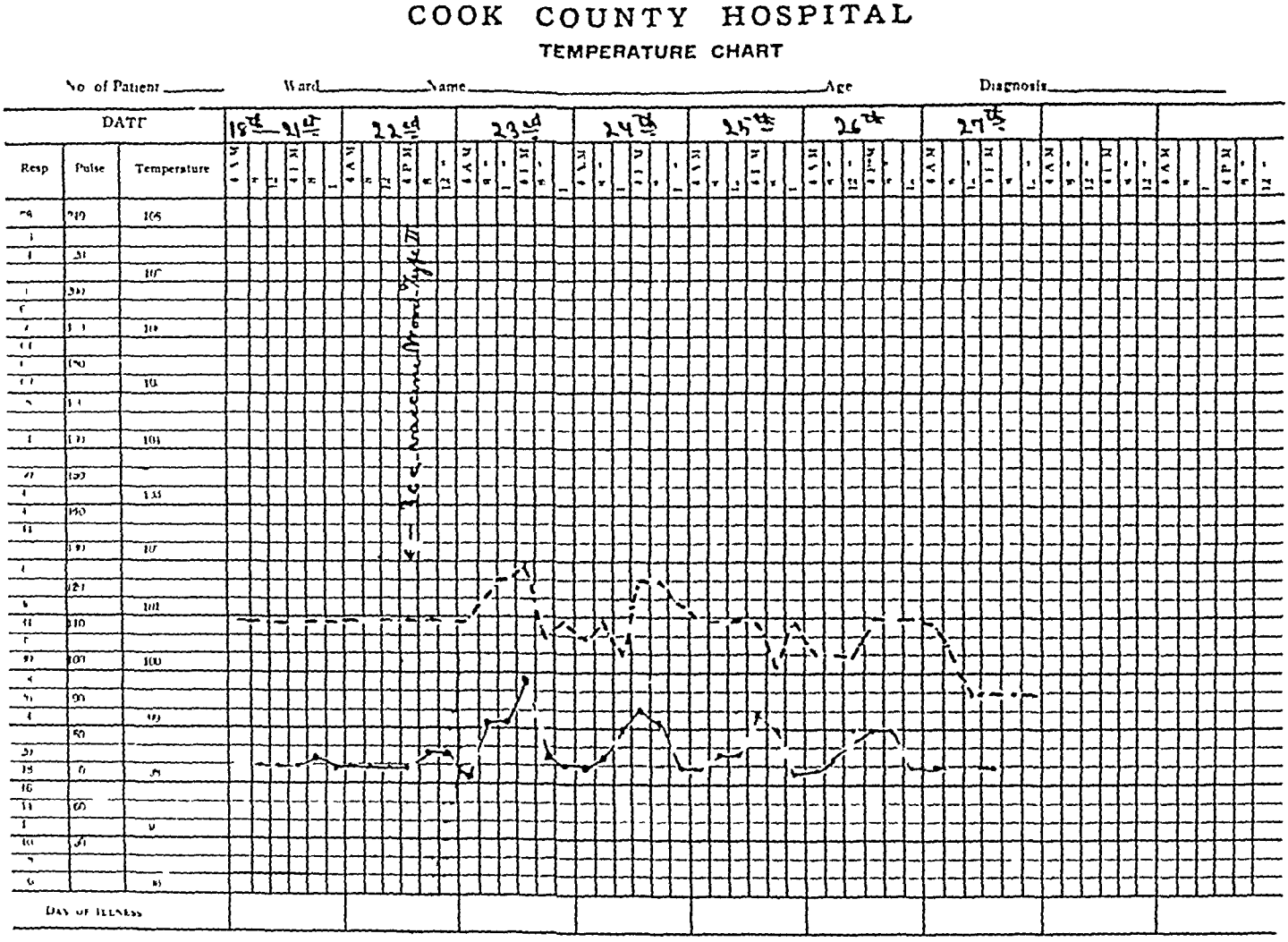


Figure 4 —Continued

hospital June 19, at which time slight dulness with increased transmission of breath and voice-sounds were still present over the apices, especially the left, but the cutaneous lesions and the abscess of the foot were apparently healed

These results indicate that in this case at least the subcutaneous injections of old cultures of blastomycetes were of undoubted benefit A local reaction in the lesions and a rise of temperature followed each injection (Fig 4, temperature chart) The intensity of the reaction was also proportionate to the quantity used, taking into consideration,

of course, the immunity which was being acquired It will also be noted that the culture of the mold from the wood was even more active than the culture obtained from the pus, and that the blastomycetic filtrate was more efficient than the suspension of the triturated growths Inoculation tests made by applying sterilized bouillon filtrate and mixed cultures triturated in lanolin to the skin which had previously been scarified, gave negative results

Further inoculation tests were made by Dr Boughton, with three suspensions of blastomycetes very kindly furnished by Dr L Hektoen Two of these organisms were from Christensen and Hektoen's two cases, and the other organisms isolated by Dr H T Ricketts A drop of each of the suspensions was placed on the skin of the forearm and the underlying skin scarified No reaction was observed Ten days later the test was repeated on the opposite arm using a minute puncture after the manner of Pirquet In twenty-four hours a distinct papule was present at the site of each inoculation and persisted for four days The control puncture showed nothing The ophthalmic test was negative Inoculation tests by both methods mentioned in several normal cases and ophthalmic tests in three normal cases gave uniformly negative results

Thanks are extended to Dr Slaymaker for permission to report the case

A CASE OF SYSTEMIC BLASTOMYCOSIS

H JACKSON, M D
CHICAGO

CASE XXV (7) —*History*—M M, a native of Croatia, entered the Cook County Hospital May 27, 1908, to the service of Dr Elliot He spoke no English and a history was obtained with difficulty His illness began one year ago with a cough and profuse expectoration which became gradually worse and was associated with considerable loss in weight Three weeks before entering the hospital a swelling with pain and tenderness appeared in the left arm followed by a swelling of the forearm and hand A week later ulcers developed in the skin of the face and other parts of the body and progressed quite rapidly

The family history was unimportant The patient had experienced no serious illness and no venereal infection He had been a farmer but after coming to this country a year ago he worked as a laborer on a railway For the previous three months he was unemployed and lived in South Chicago He drank considerable beer and whisky and smoked many cigarettes each day

On examination the patient stated that he felt well and desired to return to his native land He appeared somewhat emaciated and coughed frequently, expectorating bloody purulent sputum The respirations ranged from twenty-four to thirty the pulse from 90 to 100 per minute and the temperature from 98 6 to 101 F At this time there was on the nose an oval papillomatous lesion from 2 5 to 3 5 cm in diameter with a raised margin and a base covered with a blood stained crust under which was a quantity of brown pus A similar lesion 2 by 3 cm in diameter was present on the left cheek Other smaller lesions were present on the left arm within the area of induration previously described, over the left scapula the coccyx and on the scrotum A larger one 3 by 4 cm in diameter was found on the left buttock

No changes were found in the scalp, eyes, ears or throat Anteriorly in the neck small hard lymph nodes were palpable The indurated upper half of the left arm was extremely tender and fluctuation was obtained in its inner border The forearm and dorsum of the hand were edematous and the arm could not be moved without considerable pain (See accompanying illustration) The joints and other extremities were not affected The respirations were frequent, the supraclavicular and infraclavicular spaces deepened and the apex beat of the heart not visible Percussion elicited impaired resonance over the upper lobes on both sides, where tactile fremitus was increased Over the right side of the chest posteriorly bronchial breathing and prolonged and high-pitched expiratory sounds were heard On the left side over the upper lobe there were harsh breath-sounds but no râles

The area of cardiac dulness was not increased, and the sounds were normal The arteries were sclerotic, the pulse full, strong and regular The abdomen was distended and tympanitic, the liver and spleen not palpable At the middle of the external surface of the left testicle was a hard nodule 1 cm in diameter The epididymis also contained a hard nodule the size of a split pea The right testicle and prostate were normal No changes were found in the nervous system Examination of the blood revealed 65 per cent hemoglobin (von Fleischl) and 13,000 leukocytes, the polymorphonuclears numbered 90 4 per cent, small lymphocytes 4 8 per cent, large lymphocytes 2 per cent, eosinophils 2 per cent and basophils 0 7 per cent

Bouillon inoculated with blood from the median vein remained sterile The urine was clear, amber in color, specific gravity 1 015, reaction acid, no albumin or casts present



Case 25 —Cutaneous lesions on the nose, left cheek and shoulder, also a swollen condition of the left hand secondary to an abscess in the arm

Many blastomycetes were demonstrable in the sputum, but tubercle bacilli were absent Round and budding blastomycetes were found in the pus from the cutaneous lesions and the abscesses and afterward cultures were obtained [1]

On account of his desire to return to Croatia, he left the hospital unimproved four days after admission

1 Reference to these is made in another place

A CASE OF SYSTEMIC BLASTOMYCOSIS WITH NECROPSY

R E BECHTEL, M D, AND E R LECOUNT, M D
CHICAGO

CASE XXVI (8) —*History*—On June 28 1908, a Swede J F aged 38, was admitted to the medical service of Dr Mix, giving a history of illness beginning in the previous February with a cough, pain in the side and an expectoration occasionally blood tinged He had always been a strong robust man of good habits and when taken ill was working in a steam laundry from sixteen to eighteen hours a day Shortly after the cold began he suffered from pain in the left hip and for this went to a hospital where the thigh was incised and a large amount of pus evacuated A few days after this operation a papule appeared on the upper lip, suppurated and then became covered over with a crust Similar lesions developed on the neck chin forehead, chest, abdomen and extremities in the order given, and also on the middle finger of the right hand, following, according to his statement an abrasion During this period in the hospital, which lasted five weeks he lost greatly in both weight and strength and the cough and expectoration continued, his illness was considered as tuberculosis

When admitted to the Cook County Hospital as stated June 28 he was unable to walk, greatly emaciated and dyspneic after much conversing or other slight efforts The wounds in the thigh, two deep incisions in which drainage tubes were still present were discharging freely and necessitated his lying quietly The temperature was 101 2 F respirations 36 pulse 114 Papillomatous ulcers from 2 to 4 cm in diameter were present on the left side of the forehead, the upper lip on the right side, the back and left side of the neck and the right middle finger There were many more subcutaneous abscesses which had not ruptured located as follows two beneath the scalp one at the vertex and one over the right ear two on the back three on the abdomen one in the right axilla, three in the left arm and forearm three in the left thigh, two in the right thigh and one on the back of each leg These abscesses varied from 2 to 5 cm in diameter and were for the most part soft and fluctuating (See illustration)

Movement of the left thigh was resisted and deep pressure over the crest of the ilium caused considerable pain The cervical and axillary lymph nodes were moderately enlarged Dulness and other signs of consolidation were present over the upper part of both lungs

Treatment and Course—The disease was quite promptly recognized as blastomycosis and the organisms found and isolated in pure culture from unbroken subcutaneous abscesses Roentgen examination of the left hip revealed instead of a joint trouble, a circular defect in the left ilium slightly larger than a silver dollar, and a few weeks later by a similar examination a portion of the crest of the bone was seen lying loose in the abscess cavity During the two months from the time of admission to patient's death, August 27, the most noteworthy development in the course of the disease was the demonstration of staphylococci and *Streptococcus pyogenes* in some of the unruptured abscesses during a period of slightly more fever and profuse night sweats An antistreptococcus serum was administered without any marked benefit

There was some slight improvement during these two months, occasionally the patient was able to spend a portion of his time in a wheel chair Some of the abscesses healed after opening and cleansing with iodin The dyspnea increased toward the end and the physical examination of the chest revealed gradually increasing consolidation until all parts of both lungs were involved

The leukocytes in the blood were never over 9,000 per cubic centimeter. Occasionally small sequestra were discharged from the wound in the left hip, which remained open, over the left ilium crepitus was obtained. In the sputum with the increased pulmonary consolidation the blastomycetes became very abundant, tubercle bacilli were never found. Shortly before death the appetite failed, albumin appeared in the urine and there was some edema of the lower extremities.

The patient was given ½ grain of copper sulphate three times a day and from 30 to 150 grains of potassium iodid. Three injections of a blastomycetic vaccine given in July were followed in each instance by a local reaction and some improvement, two more in August were without benefit.

Necropsy.—The post mortem examination was held the following day after the body had been embalmed by arterial injection. The anatomic diagnosis was as follows: "ulcerative blastomycosis of the upper lobe of the left lung, disseminated blastomycosis of the lungs, liver, spleen, kidneys, adrenal, brain, subcutaneous tissues and skin, blastomycotic caries of the left iliac and right parietal bones, fibrinous and fibrous pleuritis, hyperplasia of the tracheobronchial, cervical and mesenteric lymph-glands, red marrow in the femur, emaciation, atrophy of the liver, fibrous mural endocarditis, ossification of the thyroid and costal cartilages."

The following are descriptions of the more important lesions. On the face, mostly on the right cheek but extending to the right ala of the nose, is an ulcer with elevated margins with a rather dry floor, 2.5 cm. in its longest diameter, which is downward and to the right into the yellow and rather long moustache. This is the only ulceration on the external surface of the body which has the appearance of any chronicity. The remainder are numerous, and are but little more than recently opened subcutaneous abscesses or the superficial and apparently acute destruction of the skin following such processes. The largest of these is over the left crest of the ilium about 15 cm. from the median line behind. Here is an opening 8 cm. in length with red inverted margins and packed with gauze and containing in its depths loose necrotic bone. The next largest lesion is located midway between the lower angle of the left scapula and vertebrae and consists of a purplish red oblong necrosis of the skin 6 cm. in its longest diameter, in the middle of which there is an opening into which the terminal phalanx of the middle finger is easily passed into the abscess pocket between two ribs. There are smaller abscesses, the result of a rupture or opening of subcutaneous abscesses on the entire body. There are two on the right side of the face, one on the left side of the forehead, one over the thyroid cartilage, one in front of the left elbow, four on the anterior surface of the trunk, two on the anterior surface of the left thigh, one behind the right knee and one over the middle of the left calf, these are mostly small, from 1 to 2 cm., gray or yellowish brown pus can be expressed from them, their openings are small, the skin and subcutaneous tissues are determined. In addition to these are three brownish discolorations on the abdomen on the left side, slightly elevated and fluctuating. Two similar elevated, fluctuating swellings occur, one over the base of Scarpa's triangle on the right side, and another over the internal malleolus (*right side*). The last two are not discolored externally. These swellings are about the size of hazelnuts. That in the right groin is twice the size of the other, they all contain pus more or less blood stained.

The changes in the lungs are quite alike. The two pleural sheets are adherent over a region at the apex of each lung equal to the entire palm of the hand in size. Both are fairly firm, probably from the embalming fluid in part but also from the presence of innumerable closely set gray firm regions the size of miliary tubercles or slightly larger, which are scattered throughout both lungs, more discrete below, more confluent above. In the upper part of the left lung there are a few cavities the size of a hickory-nut. There are enlarged lymph nodes about both sides of the trachea and in the cervical regions as high as the hyoid bone, there are more on the left side and larger. They vary from a buckshot



Case 26.—Photograph taken July 10, at which time the vaccine treament was instituted. Note the cutaneous lesion above the lip on the right side and the five abscesses in the chest and right forearm. Four had been recently incised, after painting with iodin

to a hazelnut in size and are firm and red on the cut surface Such lymph nodes are also found about the roots of both lungs The spleen has rounded margins, is 14 cm long, has an unchanged surface, is firm, and on cutting there is an increased amount of pulp substance seen, it is not very bloody The Malpighian bodies are distinct and there are a few grayish yellow spots slightly larger than Malpighian bodies, they are not numerous

The kidneys are slightly larger than normal and are unaltered except for scattered yellowish lesions mostly in the pyramids, from 1 to 4 mm in diameter, and the larger ones with softened centers Four or five such lesions are encountered in single vertical transverse sections The ureters and bladder are unchanged The prostate is enlarged In each lateral lobe abscesses occur containing thick gray pus On the surfaces exposed by vertical section into each lateral lobe there are a number of irregular cavities, the largest of which would contain a small pea They are more numerous in the right lobe, in which a spongy or worm eaten appearance to the surfaces is given by the number of abscesses present

Over the middle of the right parietal bone there is a necrosis of the pericranium 1 5 cm in diameter On the inside of the skull opposite this there is a slight yellowish exudate on the dura The calvarium contains but little diploe The skull here is thin, there is an extensive necrosis with sequestrum formation of the crest of the left ilium

In the brain, which was examined very carefully,[1] fourteen small lesions were found in the cerebrum and a few in the basal ganglia and deeper parts of the hemispheres, but most of them were located at the bottom of sulci or at the junction of the gray and white substance These nodules varied from 2 to 4 mm in diameter In the cerebellum there were two larger lesions, one 15 by 11 mm in the white substance between the dentate nuclei, and above the nuclei, and a second slightly smaller 4 mm posteriorly

Histological Examination.—The changes found in sections afforded no essential additional information with the exception of the liver In this organ minute focal lesions were very numerous They were about the size of renal glomeruli, located as a rule well within the lobule, and possessed the features common to all the primary minute blastomycotic lesions So far as could be ascertained the large oblong lesions in the distal portions of the renal pyramids originated from the location there of the fungi in the collecting tubules, and this explanation received some support from the occurrence of small local lesions in the cortex in which both glomeruli and tubules were involved Characteristic changes occurred in the mediastinal, inguinal and periaortic lymph-nodes, but careful search failed to reveal any in the enlarged mesenteric lymph-nodes In all the blastomycotic lymph-nodes the alterations varied from minute and widely scattered focal lesions to similar lesion so numerous and coalescent as to occupy practically all of the sections The chronic changes in the upper lobes of the lungs resembled in the extensive necrotic regions a slowly progressing chronic caseous tuberculosis In numerous sections there were encountered rounded regions of necrosis which represented occluded bronchi In the lower lobes the changes were such as have been repeatedly described, the small regions of pneumonia, the replacing of one or more alveoli in some places with single minute blastomycotic focal lesions, in other places a number of such coalescent lesions In some sections small regions of pneumonia due simply to the filling of the alveoli with leukocytes and fibrin were present, in many sections desquamated cells were abundant in the alveoli, in sections from the lower lobes some emphysema also was found The regions involved in the adrenals were highly necrotic In the prostate the sections contained simply abscess walls, and although the surrounding tissues varied, the abscess walls possessed a structure quite like

1 LeCount, E R Jour Ment Dis, 1908, xxxvi, 144

that often met in the subcutaneous abscesses Nor did sections of the brain lesions reveal anything new, on the whole there was but little tissue reaction as has been the case with the brain lesions in other bodies previously examined In some sections of the sternum made from the marrow taken at random and without reference to the presence or absence of contiguous abscesses, numerous lesions in all respects like those seen in other lymphoid tissue were present They were not coalescent nor was necrosis marked similar small focal lesions were present in the spleen in all places fungi were found in the lesions peculiar to the disease

A CASE OF SYSTEMIC BLASTOMYCOSIS

F B RILEY, M D, AND E R LECOUNT, M D
CHICAGO

CASE XXVII (9) —*History*—S C, an Italian laborer, aged 31, was admitted to the Cook County Hospital Jan 11, 1909, to the service of Dr Ormsby Through an interpreter it was learned that he had been sick for three months He suffers from persistent cough which is productive of a purulent, often blood tinged, mucus The appetite had been poor and emaciation rapid Since the onset of his illness numerous cutaneous ulcers and subcutaneous abscesses have developed He has had profuse night sweats and is now very weak

Nothing could be learned of his previous illness During the past year he lived with several other men in a small room under most unhygienic conditions

Examination—On examination the anemia and emaciation were quite pronounced A number of cutaneous lesions were distributed over the body as follows scalp and left eye, chin, back of neck, shoulder and forearm, right arm and thigh and right side of chest and back These appeared to be ruptured pustules the site of which was healing and covered with a crust or extending, producing a characteristic papillomatous ulcer Subcutaneous abscesses from 1 to 7 cm in diameter existed beneath the skin of the scalp, above the right eye, left cheek and forearm, left thigh and right forearm The left ankle was swollen and tender, especially over the internal malleolus Motion was painful and walking difficult The inguinal lymph-nodes were slightly enlarged On examination of the chest impaired resonance with diminished transmission of breath and voice sounds were present over the apex of the right lung anteriorly and the base posteriorly Both apices were dull on percussion posteriorly, and increased breath- and voice sounds were heard No râles were noted Nothing of importance was found on further examination A diagnosis of blastomycosis was made by Dr Ormsby, who had seen the patient some time before, and this was corroborated by the demonstration of the organisms in the pus and also in the sputum The pulse was 80, temperature 98 6 F, respirations 24 Although very weak the patient was able to get up and move about the ward A high evening temperature developed in the next few days, and on January 19 the patient was compelled to remain in bed because of pain in the left ankle An examination of the blood at this time showed white cells 8,600 per cubic centimeter, red cells 4,464,000, hemoglobin 72 per cent Two days later the leukocytes numbered 10,800 per cubic millimeter A differential count showed the following polymorphonuclears 87 per cent, small mononucleais 2 35 per cent large mononuclears 7 per cent, eosinophils 1 7 per cent, transitionals 1 1 per cent, myelocytes 0 6 per cent An examination of the urine also was made but nothing abnormal found

Treatment and Course—The patient was given potassium iodid in doses from 15 to 90 grains per day during his stay in the hospital Larger doses could not be given because of intolerance

From January 23 to February 25 injections of vaccine prepared from the filtrate of old bouillon cultures of blastomycetes were given in doses varying from 2 to 10 minims During this time the patient improved considerably, the pain in the ankle gradually subsided, the pulse and temperature became normal and several large abscesses were opened and began to heal At this time 2 c c of the filtrate were given and was followed by a rise in temperature to 101 and an exacerbation of the local lesions The temperature gradually decreased to 99 6 F, and on March 2 and 3 two more injections of 5 minims each were given followed by slight rise in temperature but no improvement The injections were discon

tinued at this time The temperature a few days later became irregular and high, rising at times to 103 new abscesses appeared and the old ones increased in size The general condition gradually became worse and the cough increased in severity until April 11 when death occurred During the last few days of the patient's illness a rigidity of the neck slight strabismus, exaggerated reflexes, and a positive Babinski sign were noted

Necropsy—The post-mortem examination made April 11 concluded with the following anatomical diagnosis chronic blastomycosis of the upper lobe of the left lung, erosion of the third and fourth thoracic vertebræ blastomycotic abscess of the root of the right lung disseminated miliary blastomycosis of the spleen and liver blastomycotic basilar meningitis abscesses of the prostate, peritonsillar tissues epididymis skin (with ulcers) and subcutaneous tissues caries of the foot-bones multiple intercostal subpleural abscesses blastomycotic tracheobronchial lymphadenitis Fibrinous and fibrous pleuritis fibrous peritonitis emaciation and anemia brown atrophy of the heart atrophy of the liver, slight sclerosis of the aorta and coronaries emphysema of the lungs, ossification of the thyroid cartilage

Some parts of the description of these alterations are of considerable value in giving to the reader clear notions of the disease this pertains especially to the description of the exterior of the body

"The body is that of a much emaciated man with sunken abdomen He measures 166 cm in length His hair is black and there is a sparse black beard The ears are small and pinched There are crusted lesions on the body and face as follows over the right eye on the bridge of the nose, the left eye including the lids over the left zygomatic arch on the chin in the median line in front of the right ear and 2 5 cm outside of the left angle of the mouth The largest is that involving the left eye this is the size of a half dollar the next largest is that between the eyebrows then in order are the lesions on the chin the outer angle of the mouth and over the right eye There are two lesions over the right ear covered by crusts All lesions when pressed on exude a thick gray pus A nodule is felt in the neck behind the right sternocleidomastoid the size of a walnut There are six crusted lesions at the posterior margin of this muscle, behind on the neck in the median line just below the spinous process of the first cervical vertebra and two fingers' breadth below this, all the size of a dime Fluctuation can be made out behind the mastoid process on the left side, the region involved is the size of the terminal phalanx of the thumb, it extends for two fingers below this and a little to the left There is one small crusted ulcer on the back over the spine of the left scapula a second one over the middle of the right scapula and a third lesion opposite the third thoracic vertebra a fourth occurs over the angle of the tenth rib on the left side All these lesions are flat, little more than pigmented scaling spots Many other lesions are present over the rest of the body Of these eight are on the trunk seven on the upper extremities and eight on the lower extremities One of the latter just below the right knee is an abscess which has ruptured The skin around it is covered by a brown crust, an incision into this demonstrates connection with an eroded bone The abscess is oblong 8 cm long and lying along the outer aspect of the tibia There is another lesion over the right thigh one hand's breadth below the anterior superior spine It is 4 cm across, circular in outline elevated, fluctuates and the skin over it is not changed

"The circumference of the trunk at the navel is 59 cm The right arm may be spanned by the thumb and middle finger, as may also the legs The right thigh in its middle measures 28 cm"

The alterations in the lungs were in many respects like those repeatedly noted "The right lung weighs 555 gm It floats well above the surface, it is mottled externally with brownish irregular spots which are a little sunken and most of which are 3 to 4 mm in size Deep in the fissure between the lower and upper lobes is a patch of greenish exudate the size of a finger-nail The

diaphragmatic surface is cupped and brown in color The lung is crepitant throughout The borders are pink and gray, a little rounded and elastic In the main bronchus there is a mucopus, the lining is smooth and the pus is blood stained On section there is exposed near the root an abscess surrounded by dense tissue, the contents of which are the same as the other abscesses, a thick gray green The cavity is small and would admit an object the size of a pea In the lower lobe a grayish region of consolidation is seen, this is elevated above the cut surface It is 2 cm long, has a grayish yellow irregular margin and is located about the middle of the lobe There are practically no changes in the remainder of the lung The lung contains bright red blood The left lung weighs 455 gm On removal it is torn and the torn surface is twice the size of a dollar There are adhesions at the apex where this torn region lay along the left side of the upper three thoracic vertebrae On the whole the left lung externally is similar to the right It is of a light feathery consistency and there are small, pea sized flat black, firm elevations of coal deposits on the lower lobe in the fissure In the region of the apex the lung is somewhat consolidated On section the cut surface contains a good deal of bright red blood The bronchi are like those of the right lung as regards their content The lung under the torn region is dark and beset with minute pin-head-sized regions of softening The region of consolidation about the torn apex is 10 cm in diameter

"There was a small abscess 3 mm in diameter in the left lobe of the prostate In the globus major of the left epididymis there was another small abscess"

More interesting than any of these were the changes in the brain and meninges and of these the following record was made "There are two spots in the dura mater over the anterior portion of the left parietal lobe the size of a penny, and corresponding to them the bone is pink Over the base of the brain there is a thick gray pus or fibrinopurulent exudate, thickest about the circle of Willis, where the chiasma and corpora mammillaria are completely hidden This exudate extends out into the beginning of the Sylvian fissure back over the pons and medulla and for a distance equal to the width of two fingers From these structures it gradually thins out on the inferior surface of the cerebellum When sectioned horizontally there are exposed in the fundi of the sulci aggregated, and in places single, minute gray regions, some scarcely visible, others, especially on the right side, are so large and numerous as to form a marble sized lesion All of these deeper lesions are but extensions of the external process and in none of the sections made are there deeply placed focal lesions which require an embolism of fungi to explain their occurrence The dura covering the basilar groove of the occipital bone is roughened for an area the size of a split pea, the bone beneath is unchanged" From the cerebrospinal fluid and from two of the subcutaneous abscesses the blastomycetes were recovered in pure culture In cover-glass preparations of the purulent material used no bacteria were found The peritoneal fluid and the blood of the heart proved to be sterile

Histological Examination.—A careful examination of the various viscera failed to reveal any lesions in the internal organs other than those noted in the post-mortem examination The usual changes were demonstrable in the chronic blastomycosis of the left apex, the peritonsillar and prostatic abscesses, possessed walls similar to the subcutaneous abscesses The abscess in the peritonsillar tissues in the sections examined apparently had not originated in the lymph node, for the immediately adjacent tissue was striated muscle, nor did the sections support an origin in the tonsil In the tracheobronchial lymph nodes the lesions were small and in all other respects like those found in other cases In the sections of the dura over the clivus of the occipital bone both fungi and giant-cells were found, but the minute focal lesions were pressed out flat and lay between or on the heavy fibers of the dura much like the similarly compressed "miliary abscesses" seen in the thickened pleura of other cases

A CASE OF SYSTEMIC BLASTOMYCOSIS

J S EISENTAEDT, M D, AND T H BOUGHTON, M D
CHICAGO LAWRENCE, KAN

CASE XXVIII (10)—*History*—A F man aged 19 entered the hospital April 15, 1909 to the service of Dr Grimsby He stated on admission that ten months ago he came from Bohemia where he lived on a farm and has since worked as a tailor in a sweat shop on the south side of Chicago His illness began eight weeks ago at which time he coughed much expectorating a muco purulent material which often was tinged with blood Three weeks later a small painful swelling developed on the back of the right hand This has since increased in size, and fluctuation has developed At about the same time a tenderness in the right testicle was experienced On other parts of the body there developed successively a number of cutaneous and subcutaneous abscesses The patient has lost about 20 pounds in weight since the onset and is at present very weak and is unable to work

Examination—Shortly after admission the temperature was normal, pulse 90, respirations 24 Emaciation was quite marked and abscesses varying in diameter from ½ to 3 inches were distributed as follows on the forehead, two on the right side of the head and neck, one over the left deltoid region and one in the sixth rib, two on each arm one in the left calf and one just above the heel on the same side The left globus major was somewhat enlarged and quite tender A slight dulness was noted over the right lung posteriorly being more marked over the upper lobe, where occasional moist râles, harsh breath sounds and increased vocal fremitus were heard Prolonged expiratory breath sounds over the apices were also found The heart was normal in size and a soft systolic murmur was heard over a limited area at the apex

The case was at once recognized as systemic blastomycosis and, as expected, many organisms were found in a smear made from pus aspirated from one of the abscesses The organisms were also subsequently obtained in pure cultures

In an examination of the sputum, which was bloody, no tubercle bacilli were found, but blastomycetes were abundant These were also found in the sedi ment of several centrifuged specimens of urine which otherwise were normal One week after admission the patient was anesthetized and all of the abscesses were evacuated and swabbed with tincture of iodin The abscess of the back of the hand was found to be associated with necrosis of the third and fourth meta carpal bones and these lesions are quite evident in a roentgenogram taken at that time Immediately after the operation the temperature, which previ ously had been normal, rose to 100 4 F, and this represented the high tem perature for the next few days, during which time the patient complained of a pain in the back April 25 a profuse nasal hemorrhage occurred In order to determine whether this patient would react to the injection of a blastomycotic vaccine in a manner similar to other cases, 1 c c of a filtrate of bouillon culture of blastomycetes was injected subcutaneously, April 27 The temperature on previous days between 4 and 8 p m had been 100 4 F, associated with a pulse of 100, respiration 22 On the 28th, one day after the injection, the temperature was 100 6 F at noon and at 4 p m was 102 6 F, pulse 108, respirations 24 On the following day the high temperature was again 100 4 F and has remained at about this point Increased pain or changes in local lesions were not noticed

A differential count of the leukocytes made before the injection showed poly morphonuclears 68 per cent, large mononuclears 17 per cent, small mononu clears 13 per cent, eosinophils 1 per cent Another examination made May 3

resulted as follows leukocytes 7,000 per cubic millimeter Of these the polymorphonuclears constituted 62 per cent , large mononuclears 13 per cent , small mononuclears 22 per cent , and eosinophils 3 per cent The hemoglobin by both Dare and Sahli Gower instruments was 57 per cent

Treatment—Potassium iodid was administered in doses which were gradually increased to the extent that on May 3 the patient was receiving 70 grains three times a day He was kept in the open air as much as possible and a nutritious diet given In spite of this the patient seemed to grow weaker and on May 5 six new abscesses about an inch in diameter were found to have developed in the extremities, and the patient complained of pain in the back and left foot In the latter a small, tender swelling about the second metatarsophalangeal junction was found Roentgengrams were taken of this region and also of the right hand and the sixth rib, in which lesions existed as described

On July 12, 1909, it was noted that the patient's general condition was somewhat less favorable A number of abscesses had healed and new ones had occurred as follows one over the right scapula, one in the right forearm just above the wrist, one in the left arm, and one under the right eye The right shoulder was very painful and on close examination the acromial process of the scapula was found to be involved to the extent that displacement was possible The right thigh also was very painful, but no superficial abscess could be found The lungs were involved to a greater extent than before, but no other visceral changes were noted From this time the patient grew gradually weaker and many new abscesses appeared Death occurred December 13 The necropsy report is as follows

Necropsy—The body is that of a young adult white male, 158 cm in length, considerably emaciated The hair in the back of the scalp is worn off On the left side of the forehead near the hair-line is a small triangular scar 2 cm in diameter The eyes are sunken The facial muscles are atrophied Just below the left orbit is a superficial skin lesion, about 1 5 cm in length, slightly elevated and crusted A similar lesion, somewhat hemorrhagic, is present near the right ala of the nose

Just above the tip of the left shoulder is an ulcerating skin lesion measuring 5 by 3 5 cm The margins are purplish, contain scar tissue and are slightly elevated, the floor of the ulcer consists of the ligaments of the shoulder joint, and an area about 1 by 2 cm seems to connect with the shoulder joint The anterior aspect of the left shoulder shows a soft, egg shaped swelling 2 cm high which fluctuates On the right arm half way between elbow and shoulder on the lateral aspect is an ulcer measuring about 4 by 5 cm The margins are scarcely raised, and in the floor exposed muscular tissue may be seen The right elbow can be extended to an angle of about 120 degrees, crepitus is felt, a soft fluctuating mass is present on the posterior surface just above the olecranon On the volar surface of the right forearm about 5 cm below the elbow is an elongated pigmented scar A few other small scars appear lower down on the forearm Motion of the wrist is much restricted in all directions, and crepitus can be felt and heard On the back of the right hand, over the middle metacarpal is an ulcerated area about 1 by 3 cm , communicating with the subcutaneous and interosseous tissue, and on pressure a small amount of mucoid yellowish material can be expressed About the base of the first metacarpal, a purple fluctuating area may be felt On the radial aspect of the left forearm about 6 cm below the elbow is a crusted skin lesion about 2 by 0 5 cm

Posteriorly just above the spine of the right scapula is a skin lesion about 1 5 cm in diameter communicating with the deeper tissue, from which a small amount of yellowish mucoid material can be expressed Just below the level of the twelfth rib at the right of the spine are two small fistulous openings in the skin surrounded by a purple areola, from which a small amount of necrotic material can be expressed On the right side, about 1 cm above the level of

the iliac crest is a small ulcerated lesion from which a yellowish cloudy liquid escapes Over the sacrum is a large decubital ulcer measuring 12 by 4 cm Both hips are ankylosed in a semiflexed position, and in the left hip crepitus is evident

A muscular contracture holds both knees in a semiflexed position About the right knee on the anterior surface are many areas of cicatricial tissue of varying sizes Just above the left patella is a superficial skin lesion measuring 4 by 2 cm covered by thin papery scales of epidermis Near this and to the left of it is a similar lesion about 1 cm in diameter On the anterolateral aspect of the left leg about 15 cm below the knee is a small superficial ulcer The anterior borders of both tibiae are very sharp and somewhat curved laterally The left foot, especially on the dorsum, is swollen and puffy Between the first and second toes of the left foot is a ragged ulcer about 1 cm in diameter from which grumous material can be expressed

When the abdominal cavity is opened, the peritoneum is smooth and shining The omentum is devoid of fat, and is rolled up transversely The lower border of the liver lies about one finger's breadth above the costal margin in the mammary line The appendix lies over the brim of the pelvis, it is about 5 cm in length, is free from adhesions and appears normal It has a mesentery of its own for its entire length

The right pleural cavity has posteriorly and laterally a considerable number of easily broken adhesions The lung is also somewhat adherent to the diaphragm This cavity contains no fluid The left pleural cavity is free from adhesions and fluid, except at the level of the fifth rib where it is firmly adherent to the chest wall At the site of this adhesion, the fifth rib for a distance of about 2 cm is considerably eroded, the osseous tissue being practically absent

When the pericardial cavity is opened the pericardium is smooth and shining, and contains a small amount of free fluid The heart is rather small On the anterior surface of the right ventricle is an area about 1 by 2 cm which is light in color, slightly elevated and somewhat thickened The mitral orifice admits the tips of two fingers The left ventricle is firmly contracted The musculature is rather pale The wall measures 15 mm The mitral valve is normal in appearance The foramen ovale is closed The aortic orifice admits one finger readily The aortic leaflets are normal in appearance, as is also the base of the aorta The tricuspid valve appears normal as do the pulmonary artery and pulmonic valve The area referred to on the anterior wall of the right ventricle is confined chiefly to the pericardium, but a small whitish area extends into the musculature The weight of the heart is 200 gm

The spleen weighs 200 gm It is somewhat enlarged the surface is smooth, the consistency is firm Near the upper pole on the anterior and lateral surface is a slight constriction marking off a small lobule The surface is purplish, the capsule strips with difficulty On section, the color is redder than usual, the markings are fairly distinct

The tongue, trachea and esophagus appear normal

The right lung weighs 450 gm The lung collapses somewhat, it crepitates feebly It shows evidences of fibrous adhesions on all except the anterior surface The upper lobe feels firm, but no small nodules can be felt On section the lung is light red, on pressure a small amount of frothy fluid can be expressed At the base of the upper lobe is an area which is firm, dark purple and airless On pressure, no fluid escapes from the alveoli, but a small amount of purulent material from the mouths of the smaller bronchi At the upper edge of this area is a pea sized cavity containing a small amount of yellow mucoid material The left lung weighs 400 gm The surface is free from adhesions except at the base, and at the lower margin of the upper lobe Very little crepitation is felt On section, the lung is light in color, and on pressure a very slight amount of frothy fluid may be expressed

The pancreas is normal in appearance and consistency except that the lobules are rather poorly marked On section it shows no pathological change

The liver with the gall-bladder weighs 1,700 gm. The gall-bladder contains a moderate amount of dark bile and no stones. The surface of the liver is smooth, its color is mottled pinkish. On section the color is a rather muddy yellowish brown, the markings are fairly distinct, on pressure a small amount of bloody fluid is expressed.

The stomach is about normal in size. The rugae are well marked. The mucosa appears normal.

The kidneys together weigh 450 gm. The left kidney is the larger of the two. The surface is pink, and is somewhat irregular. The cut edges do not bulge. The capsule strips readily. The color is pale. The ratio of cortex to medulla is 12: 18. The markings are indistinct. The right kidney is smaller, but otherwise corresponds to the description of the left.

At the level of the fourth lumbar vertebra on both sides are found abscesses. On the right side the abscess has burrowed laterally about 9 cm. beneath the posterior abdominal wall. Inferiorly, it has burrowed far below Poupart's ligament. On the left side the abscess has burrowed laterally but a short distance. Inferiorly it has burrowed far below Poupart's ligament. Both abscesses have pointed posteriorly. The right side of the body of the fourth lumbar vertebra is somewhat roughened. On chiseling into it, an abscess cavity is encountered which occupies about one third of the body of the vertebra. It contains a yellow mucoid material. The third and fifth lumbar vertebrae show no gross lesions. The cartilage between the fourth and fifth lumbar vertebrae is quite soft.

The large intestine is negative. The lower portion of the ilium shows a number of small congested areas, but otherwise no change.

Anatomical Diagnosis.—Generalized blastomycosis involving skin and subcutaneous tissue in many regions, left shoulder, right elbow, right wrist, left hip, fourth lumbar vertebra, right lung and left fifth rib, adhesive pleuritis—bilateral, passive congestion of liver, acute splenitis, chronic parenchymatous nephritis, bilateral psoas abscesses (blastomycotic), consolidation at base of right upper lobe with small abscess (blastomycotic).

Blastomycetes were recovered in pure culture from the cavity in the right lung, and were found in smears of the pus from all of the abscesses, but only in the lung were they numerous. The smears were examined for tubercle bacilli, but none were found. The microscopical examination of the tissues will be reported later.

A CASE OF SYSTEMIC BLASTOMYCOSIS

PHILIP FRANK SHAFFNER, M D
CHICAGO

CASE XXIX (11)—*History*—J D, aged 30, Hungarian, has resided in this country for about two years and speaks no English He was first employed in West Pullman, Ill for a short time, working in a foundry He next worked in the car shops, assorting and piling on trucks the various kinds of wood Patient declares that his hygienic conditions have always been good (good ventilation, no dampness, etc) and that he has slept alone in a room which he describes as being dry and large enough for two beds There is no history of any other occupant of the house having had the same trouble as his

Patient dates his illness from Oct 1, 1909 Illness started with a bad cold, a severe cough and pain in left side He does not know if he had fever Three weeks later he began to cough up blood, at times in large amounts, and he has continued to have bloody expectorations daily, two or three in number since then He became dyspneic shortly after the onset of his cough He does not know how much weight he has lost but says he weighed 160 pounds at one time before his illness started At present he weighs about 110 or 120 pounds He has had no night sweats and thinks he has had no fever whatever during his sickness His appetite has been good No history of bloody or abnormal number of stools can be obtained Patient states he has not vomited or suffered any epigastric discomfort There has been no pain on urination but he complains of a constant pain in the back which has been so severe as to incapacitate him and at times keep him from sleeping Coughing has now ceased for the past two weeks

About six weeks ago the present lesion over the right eye appeared A few days later a second similar lesion was noted over the left eye These were followed in rapid succession by numerous abscesses elsewhere on the face extremities and trunk, and were accompanied by the usual signs and symptoms of any ordinary localized skin infection He has been getting weaker all the time and now is unable even to sit up in bed

The patient entered the Cook County Hospital Dec 2 1909, and was admitted to the Tuberculosis Hospital with the diagnosis of pulmonary tuberculosis It was while there that he developed the skin lesions which aroused the curiosity of the intern who consulted Dr O S Ormsby as to their nature The latter made a diagnosis of systemic blastomycosis and patient was transferred to the Skin and Venereal Ward on Dr Ormsby's service While he was at the Tuberculosis Hospital, three sputum examinations were made for the presence of the tubercle bacilli but with negative results

The man's family history throws no light on the etiology He is married and has two children living and well Patient drinks several whiskies and about one pint of beer daily Smokes moderately Had gonorrhea five years ago Three years ago he was ill for eight months with a disease of unknown nature He says that he felt weak, coughed, had no night sweats, and lost little weight At this time he had no skin lesions of any sort

Examination—Physical examination, March 5, 1910, elicited the following The patient is very much emaciated, highly anemic with a lemon tint to skin, he appears very ill, and is too weak to move about No cyanosis or dyspnea No edema of extremities, etc

Multiple abscesses are scattered diffusely over body These are of two types Those of the face and scalp are crusted, globular, about the size of a large hickory-nut, brownish red and surrounded by a slight inflammatory zone Some have ruptured, and exude a thick yellowish mucoid material These lesions are scat-

tered diffusely about the face and scalp Those of skin of extremities and trunk are not crusted, the overlying skin is but slightly inflamed even in the case of the large abscesses and those of great tension All are painful on pressure and prevent the patient from moving about Anteriorly on the chest wall over the seventh rib at its junction to the sternum is a small fluctuating abscess apparently connected with that rib Likewise an abscess over the region of the sixth dorsal vertebra appears to have communication with the lateral process of that vertebra

The tongue is badly coated, there is moderate pyorrhea alveolaris, the breath is foul and heavy, tonsils moderately enlarged A large submaxillary lymph-node about the size of a small hen's egg exists on the left side of the neck, it fluctuates and is tense

Litten's sign is absent on right side of the chest There is marked emaciation of chest wall with depression of superclavicular spaces Motion is restricted in both sides

Dulness is obtained on the left side posteriorly over lower part of upper lobe and entire lower lobe, and also impaired resonance of right apex, and over entire left lung anteriorly

Bronchial breathing with fine crepitant râles, increased vocal and tactile fremitus, and exaggerated whispered sounds are noted over the dull area posteriorly on the left side Mucous clicks are heard over the left lower lobe posteriorly

Anteriorly a few râles are heard in both axillae, and also fine crepitant râles in both apices The râles of the dull area on the left side are few in number — the breath sounds are harsh and roughened with a moderate prolongation of the expiratory murmur The findings on percussion suggest an advanced pulmonary tuberculosis, while the auscultatory findings would indicate an incipient stage of the disease No signs of abscess cavity can be elicited The heart is enlarged a little to the left, and on auscultation an apical systolic murmur can be heard

The pulse is small in volume and moderate in tension, with a tendency toward dicrotism

The spleen, liver and kidneys are not palpable

Except for some adenopathy of the inguinal lymph nodes, further examination was negative

The patient's temperature ranged from 98 4 to 103 4 F, with periods of normal temperature for days at a time No characteristic evening rise or morning subnormal temperature is recorded

The respiratory rate varied from 20 to 36 according to the temperature

The pulse rate ranged from 86 to 110

Urine Specific gravity 1 020, acid, cloudy and amber colored, no albumin, no sugar Microscopical Few hyalin casts, few leukocytes, many urates and phosphates, no blood microscopically or chemically

No blastomycetes were found in the urine on repeated examination

Blood Hemoglobin 48 per cent (Dare and Sahli), erythrocytes 1 600,000, color index 1 46 +, white cells 22,000 Differential count polymorphonuclear neutrophils 86 per cent, eosinophils 1 per cent, mononuclears, large 9 per cent, small 4 per cent, moderate poikilocytosis

Blood cultures were negative

Sputum was negative for tubercle bacilli and blastomycetes

Feces Negative for blood (Weber)

The Wassermann test was kindly made by Dr J H Waugh, and was negative

A subcutaneous injection of tuberculin (Koch's old), 0 02 mg, was given, increasing 0 04 mg each dose for three successive doses at intervals of three days This was done during the period of apyrexia — after the last dose, the temperature was 101 + F and remained about the same for the following few

days, when it again became normal Because of the irregular temperature curve, little importance as to the presence or absence of tuberculosis can be attached to the results

A direct smear from one of the intact abscesses mounted in 20 per cent potassium hydroxid showed the characteristic morphology of the blastomycetes — budding forms, etc Cultures from two skin lesions showed the organism in pure culture

In summarizing it is noted that this case resembles pulmonary tuberculosis in the following onset with cough hemoptysis, loss of weight and strength, marked emaciation, lung findings

In this regard it is noted that the auscultatory findings are insignificant as contrasted with those elicited on percussion, namely, an area of greatly impaired resonance will give few or no rales, etc

MULTIPLE PRIMARY MALIGNANT TUMORS

WITH A REPORT OF TWO CASES IN DOGS *

FRANK K BARTLETT, M D
CHICAGO

Multiple primary malignant tumors occurring in the same individual are comparatively rare Gotting[1] in over a thousand necropsies on malignant growths encountered only two cases, von Hansemann[2] in the same number of necropsies found five, Redlich[2] in 507 cases had two, Reichmann[2] in 711 cases also had two Several writers look on these sporadic cases as purely accidental, while others, as Adami,[3] Wooley[4] and Williams,[5] attach considerable importance to them, especially in the further consideration of the nature and etiology of malignant tumors

In the vast majority of cases, single malignant tumors originate from a single type of tissue, that is (for example), the squamous epithelium, the low cuboidal cells of the mammary acini, or the connective tissue Malignant tumors are rarely composed primarily of both a transformed epithelium and of a transformed mesoderm Until 1901, Wells[6] found but three undoubted cases of this type, including his own case, the so-called sarcocarcinomas, all of which had developed from the thyroid gland In Wells' case the primary tumor was a mixture of a carcinoma with a sarcomatous stroma, giving metastases of pure carcinoma, of pure sarcoma, and of a mixture of both Herxheimer,[7] in 1908, collected from the literature reports of twenty cases of carcinosarcomatodes occurring in man There are many cases of this sort of tumor reported in the literature that are not true carcinosarcomatodes, but are mixtures of two entirely

* From the Department of Pathology, University of Illinois
* Submitted for publication Dec 10, 1913

1 Gotting, H Zur Multiplizitat primarer Carcinome, Ztschr f Krebs forsch, 1908 1909, viii, 675

2 Quoted by Gotting (See Footnote 1)

3 Adami, J G Concerning the Causation of Cancerous and Other New Growths, Yale Med Jour, 1901, vii, 309 and 343

4 Wooley, P G Notes on Multiple Primary Tumors, Boston Med and Surg Jour, 1903, cxlviii, 1

5 Williams, R Bilateral Tumors — Malignant and Non Malignant, Lancet, London, Feb 1, 1910, p 426

6 Wells, H G Multiple Primary Malignant Tumors, Etc, Jour Path and Bacteriol, June, 1901, p 357

7 Herxheimer, G Ueber das Carcinoma Sarcomatodes und einen einschlägigen Fall des Oesophagus, Beitr z path Anat u z allg Path, 1908, xliv, 150

separate tumors, namely, a sarcoma and a carcinoma, each having originated separately and later having so thoroughly mixed with one another as to give the appearance of a tumor of single origin Such a case is reported by Spencer,[8] in which a sarcoma and adenocarcinoma occur close together in the uterus in places mixed, resembling a true sarcocarcinoma Closely allied is the case reported by Schone[9] in which there is in the thyroid both a sarcoma and a carcinoma, closely approximated, yet quite distinct from each other Nebesky's[10] case of a spindle-cell sarcoma of the uterus together with a papillary adenocarcinoma adjacent to it exemplifies a similar condition Some of these cases then are indistinctly separated multiple primary malignant tumors

It is interesting in this connection to point out that Ehrlich and Apolant,[11] by inoculating a typical adenocarcinoma through ten generations of mice finally obtained a mixed tumor the stroma of which was a spindle-cell sarcoma By the thirteenth generation all traces of the original carcinoma tissue had disappeared, leaving a sarcoma which as late as the twenty-sixth generation was still a typical sarcoma Sticker[12] reports a similar experience he inoculated a spindle-cell sarcoma into the mammary gland of a dog This resulted in the formation of a typical mammary carcinoma Like results have been reported by Lewin and by Loeb According to Ewing, in discussing the case of Ehrlich and Apolant there are four possibilities first there is a transformation of epithelial cells into the sarcomatous-appearing cells second, there is a sarcomatous element in the original tissue third, the stroma of the original tumor has become malignant and fourth, the stroma of the host has become malignant

It is the general conception at present that any one malignant tumor originates from one single primary focus, or in other words, that it is monocentric Peterson, however, from the study of serial sections of early malignant tumors, believes that a pluricentric origin is the rule rather than the exception Wooley[4] reports a case of carcinoma of both adrenals in which there are in all five primary foci of similar histological structure The proximity of these foci, three being in one adrenal, supports Peterson's stand, and makes it plausible that foci still more closely set would at a later stage in the growth of the tumor be conglomerate

8 Spencer, H R Carcinosarcoma Uteri, Tr Obst Soc London, 1905 xlvii, 235

9 Schone, G Sarkom und Karzinom in einer Schilddruse beim Hunde, Virchows Arch f path Anat, 1909, xix, 169

10 Nebesky Arch f Gynak, 1904, lxxiii, 653

11 Ehrlich, P, and Apolant, H Zur Kenntnis der Sarkomentwicklung bei Carcinom Transplantation, Centralbl f allg Path u path Anat, 1906, xvii, 513

12 Sticker, A Experimentele Erzeugung des Carcinoms beim Hunde, Arch f klin Chir 1909 xc 577

and would obscure its multicentric origin From the study of very early basal-cell carcinomas, for example, one is impressed by the diffuse area of cells, apparently all primarily on the road to malignancy In discussing his case of multiple primary carcinomas of the adrenal gland, Wooley states that the adrenal cells surrounding the small primary carcinoma foci appear to be undergoing malignant transformation, he suggests that all the normal cells about these foci are unbalanced and are tending toward malignancy It is, of course, impossible to determine in tumors beyond their early growth whether or not their initial impetus was from a monocentric or pluricentric origin One cannot exclude entirely the possibility of a local predisposition, which in a broader interpretation indicates the susceptibility of a certain tissue, that is, the skin, or fascia, or even a system of tissue including, for instance, the mammae, the ovaries, the uterus and the tubes Thiersch,[13] Adami, Bucher,[14] Warthin,[15] Wooley and others have mentioned local predisposition or susceptibility, in explanation of the occurrence of distinctly separated, though closely seated, primary malignant tumors

The fact that multiple primary malignant tumors are often bilateral and symmetrical is significant perhaps in many cases of a predisposition on the part of the tissue affected This predisposition, Williams believes is a developmental disturbance A great many instances supporting this view are to be found in the literature Zangemeister[16] found that out of thirty-nine sarcomas of the ovaries, ten were primarily bilateral Pick[17] found nine bilateral primary sarcomas out of a series of twenty-three cases of sarcomas of the ovaries Schubert[18] found three primary bilateral malignant testicular tumors out of a series of fifteen cases of malignant tumors of the testicle In a series of 114 similar cases, Kober[19] found five bilateral instances Williams points out that these malignant tumors of the testicle and ovary are more apt to be bilateral when the malignancy has developed early, and in view of this fact connects them with some developmental anomaly Ladinskie[20] describes a case of sarcoma of one ovary and of a carcinoma of the other ovary in a woman

13 Thiersch Quoted by Warthin (see Footnote 15)

14 Bucher Quoted by Warthin (see Footnote 15)

15 Warthin, A S Multiple Primary Carcinoma, Jour Am Med Assn, 1899, xxxii, 963

16 Zangemeister, D W Ueber Sarkome des Ovariums, Beitr z klin Chir, 1896, xvi, 397

17 Pick, L Zur Symptomatologie und Prognose der Sarkome des Eierstocks, Centralbl f Gynak, 1894, xviii, 940

18 Schubert Quoted by Williams, W R Natural History of Cancer, New York, William Wood & Co, 1908

19 Kober, G M Sarcoma of the Testicle Conclusions Based on One Hundred and Fourteen Cases, Am Jour Med Sc, 1899, cxvii, 535

20 Ladinskie, L J Absence of the Uterus and Vagina with Sarcoma of One Ovary and Adenocarcinoma of the Other, Am Jour Obst, 1898 xxxviii, 78

in whom both fallopian tubes and the uterus were congenitally absent In this case anomalous embryonal growth is quite conclusively associated with the development of the bilateral malignancy Rolleston and Marks,[21] out of twenty-six cases of malignant diseases of the adrenals, found that two cases were primarily bilateral Walker[22] found ten primary bilateral malignant tumors out of a series of 141 cases of malignant disease of the kidneys Kuster,[23] in the review of 607 primary malignant tumors of the kidney, found that there were thirteen primarily bilateral A very remarkable case is cited by Newton,[24] in which ten children out of a family of sixteen died of glioma of the retina before they had attained the age of 5 years Of these ten cases, seven were bilateral It is seen from the foregoing instances, which are representative only of a class of cases, that multiple primary malignant tumors may occur in paired organs, and that the percentage of such cases is too high to be considered an incidental occurrence These cases seem to indicate that a certain type of tissue may be predisposed to malignant transformation under favorable circumstances of irritation They indicate some anomaly in the embryonal development of that particular set of organs

Excluding bilateral primary malignant tumors of the ovaries, testicles, kidneys and adrenals, and also the sarcocarcinomas I have reviewed reports of ninety-five cases of multiple primary malignant tumors, among these ninety-five cases are included the list reviewed by Williams, by Wooley and by Wells In thirty of the ninety-five cases, there is no predilection for any particular system or type of tissue, twenty-seven of these thirty cases are represented by both a carcinoma and a sarcoma, while the remaining three cases are represented by only one type of malignant tumor Thirty-four of the ninety-five cases occur in the reproductive system, the mammae, the ovaries, the uterus the tubes and the vagina are included under this head, only one case in the male is reported Of these thirty-four cases, sixteen are represented by both a sarcoma and a carcinoma, while in eighteen of the thirty-four cases only one type of malignant tumor is present, that is, either a sarcoma or a carcinoma Thirteen of the ninety-five cases occur in the cutaneous tissue, two of these are each represented by a sarcoma and a carcinoma, while the remaining eleven are all represented by carcinomas Again, thirteen of the ninety-five cases occur in the gastro-intestinal tract, including the

21 Rolleston, H D, and Marks, H W J Primary Malignant Disease of the Suprarenal Bodies, Am Jour Med Sc, 1898, n, 383

22 Walker, G Sarcoma of the Kidney in Children, Etc, Ann Surg, 1898, xxvi, 529

23 Kuster Ueber Neubildungen der Niere und ihre Behandlung, Centralbl f d Krankh d Harn u sex Org, 1897, viii, 583

24 Newton, R E Glioma of Retina, Australasian Med Gaz, 1902, x, 236

esophagus, the stomach, the intestines, the liver and the pancreas Of these thirteen cases, four are represented by two types of tumors, namely, a sarcoma and a carcinoma, while the remaining nine cases are represented by but one type of malignant tumor There are three cases of multiple primary malignant tumors in bones, one case in the central nervous system, and one case in the glands of internal secretion In all ninety-five cases there are but four instances wherein more than two primary malignant tumors occur in the same individual

Briefly, then, it is evident that two-thirds of all these cases of multiple primary malignant tumors are confined to systems, while but one-third of the cases are miscellaneously distributed A comparison may be drawn between the foregoing distribution of multiple malignant tumors and the multiple benign tumors, especially the subcutaneous neurofibromas, lipomas, ecchondromas and fibromas, the occurrence of which undoubtedly indicates some predisposition on the part of the tissue involved We know that each of these multiple benign tumors is primary, because metastatic distribution can be ruled out The oft-repeated statement can be reiterated here that "no sharp line of division is possible between the benign tumors and the malignant tumors" This fact, together with the foregoing statistics, indicates and indirectly confirms that multiple primary malignant tumors are, in certain cases at least, due to the existence of some susceptibility on the part of the tissue or system of tissues involved Adami points out that in the female the involution of all the reproductive tissues at the climacteric is in some way connected with the very frequent occurrence of multiple tumors in this particular system Williams believes that since the female reproductive system attains physiological maturity earlier than the rest of the body, its chances to develop malignancy are greater

The type of malignant growth, as seen from the before-mentioned cases, is not so highly important in the consideration of multiple primary malignant tumors as is the system or tissue in which these tumors occur Many factors, such as irritation, misplaced embryonal rests, climacteric and senile involution, parasites or infection may be involved in the lighting up of these system tumors But behind these indirect causes which, boiled down, mean "irritation," there is, as far as can be determined, something else which in the interpretation of to-day is called a "biologic" disturbance

This biologic disturbance may lead to a local, to a tissue, to a system, or to a general predisposition to malignant tumor growth The recent work of Miss Sleye[25] at the University of Chicago in collaboration with Dr Wells, has been the most extensive research yet done on experimental

25 Sleye, M Statistics to be published later

heredity of cancer in mice Their work goes to show that an infective focus of long standing is much more apt to produce a malignant tumor in a cancerous strain of mice than in a non-cancerous strain, in the latter an infective nodule, or perhaps a benign tumor, instead of a beginning malignant focus may result from such an irritation They further point out that multiple primary malignant tumors are much more frequent in these cancerous strains of mice than in the non-cancerous strains A sarcoma is as apt to crop out in these mice as is a carcinoma It appears that in the cancer strains there is a definite hereditary predisposition and that this predisposition is general, including all types of tissue and all parts of the body The best workers in the field of heredity in human cancer have so far failed to find any definite proof of a hereditary predisposition to malignancy

CASE-REPORTS

The two cases of multiple primary malignant growths reported below occurred in Dogs A and B, both of which were brought to the laboratory for ordinary class-work

CASE 1—Dog A is an old male dog weighing approximately 60 pounds In an attempt to insert a tracheal cannula, a large diffusely infiltrating and very vascular growth was encountered in the region of the thyroid (Fig 1)

Thyroid The thyroid is firmly embedded in the surrounding soft tissues almost completely encircling the trachea but not being adherent to or penetrating the latter In places an irregular thin capsule is present, but it is not defining to the tumor mass The tumor, which is very irregular, is 13 cm long and 9 cm wide the larger portion lying on the right side The large veins coursing over and leaving the tumor are almost all filled with papillous worm-like tumor thrombi some of which attain a length of 5 cm The regional lymph-nodes are not involved by metastases, though on the left side two or three are incorporated in the tumor mass

The cut surface of the tumor is very fibrous with soft centers or nests of cells in it In places where the stroma is scarce, the tumor is soft and grossly like a small round cell sarcoma In either side is a small, necrotic and old hemorrhagic region

Lungs The lungs are the seat of numerous whitish gray nodules, spherical in outline They range from 1 mm to 2 cm in diameter and are about eighty in number

Mediastinum In the anterior mediastinum there is a nodule the size of a pigeon egg, pedunculated and encapsulated Two thirds of it is gelatinous, the remainder is solid

Adrenals The right adrenal is replaced by a slightly irregular spherical tumor, 5 5 cm in diameter (Fig 2) It is surrounded by a thin fibrous capsule in which there are a few small veins The color through the capsule is distinctly a dirty yellow and brown, quite similar to the ordinary renal hypernephroma It is distinctly separated from the right kidney by a distance of 1 5 cm At its inner border there is a nodule, 0 5 cm in diameter, protruding from it, for a distance of 0 7 cm The whole tumor is easily enucleated from the surrounding tissue This adrenal tumor was cut into sagittal sections, 0 7 cm apart The section through the center reveals a surface that is for the most part necrotic, although it is rather firm It is brownish-gray with irregular streaks of yellow through it, at the edges the mottling is of a bright reddish brown as though

from fresh hemorrhages At the lower inner pole of the tumor, included within the capsule yet separated from the rest of the mass by a thin fibrous septum, is a crescent-shaped peripheral cap of tissue, 0 7 cm thick and 4 5 cm long This is yellow and solid, it is quite unlike the rest of the cut surface Other surfaces made by the sagittal sections reveal a similar condition, as just described, except that near the surface of the tumor the necrotic appearance fades and the yellow tissue, mottled with red, is in preponderance

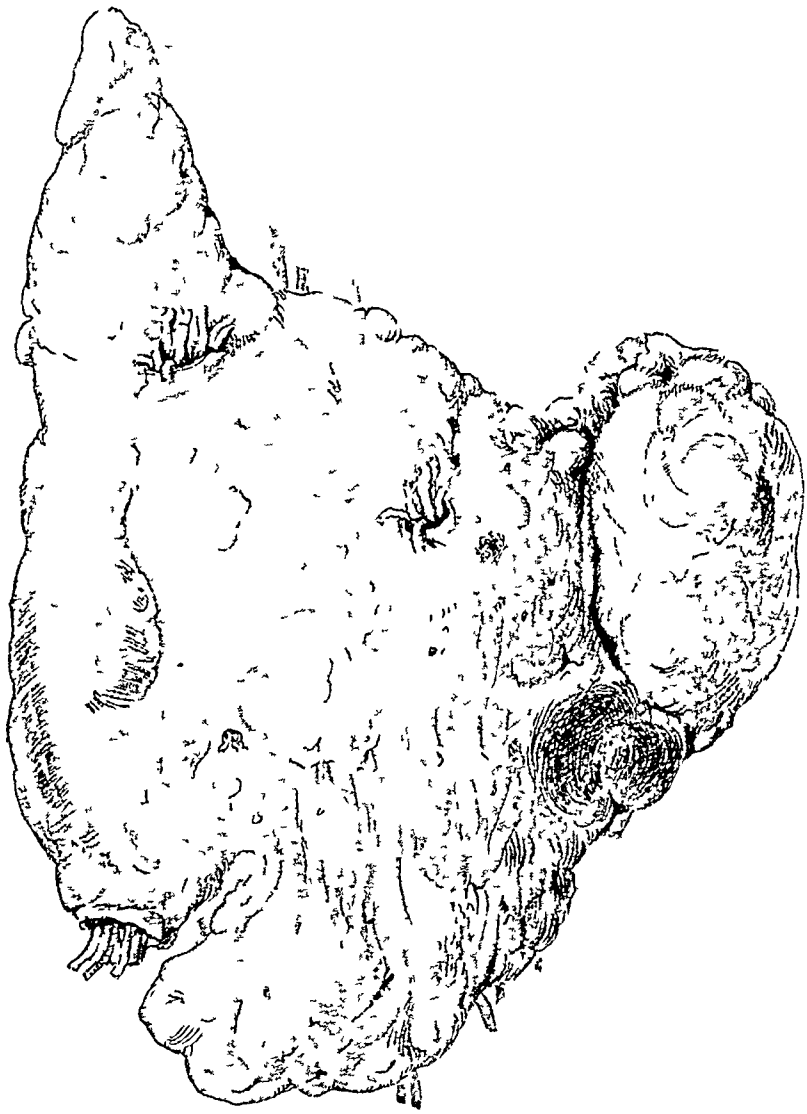


Fig 1 —(Two thirds natural size)—Dog A Carcinoma of the thyroid The large veins coursing over and leaving the tumor are numerous and full of papillomatous tumor thrombi The capsule is thin and ill defining

The left adrenal is replaced by a spherical tumor 4 cm in diameter, surrounded by a thin capsule and entirely separated from the kidney (Fig 2) It is easily enucleated from the surrounding tissue It is separated from the right adrenal tumor by a space of 4 cm , and no connection between the two is visible grossly This tumor is a little firmer than the right one On surfaces made by sectioning, it is seen to be beset throughout by small whirls of yellowish tissue, similar to adrenal cortex in color These whirls range from 1 mm to 1 5 cm in diameter The largest ones are seminecrotic

Kidneys Both kidneys are of the congenital multicystic type, the cysts being very numerous throughout both kidneys and ranging from 1 mm to 3 cm in diameter (Fig 2)

Liver The liver is normal except for a small 1 5 cm cavernous hemangioma

General The rest of the body on careful examination reveals nothing of importance in connection with these tumors

Microscopic All the microscopical sections are stained with hematoxylin and eosin

Dog A Sections examined from various places in the thyroid reveal an adenocarcinoma, the alveoli of which are well formed in places and contain colloid The majority of the alveoli are irregular, incomplete and without colloid In the regions in which alveoli are present the stroma is thick and fibrous In the softer and non fibrous portions of the tumor, the microscopic picture is that

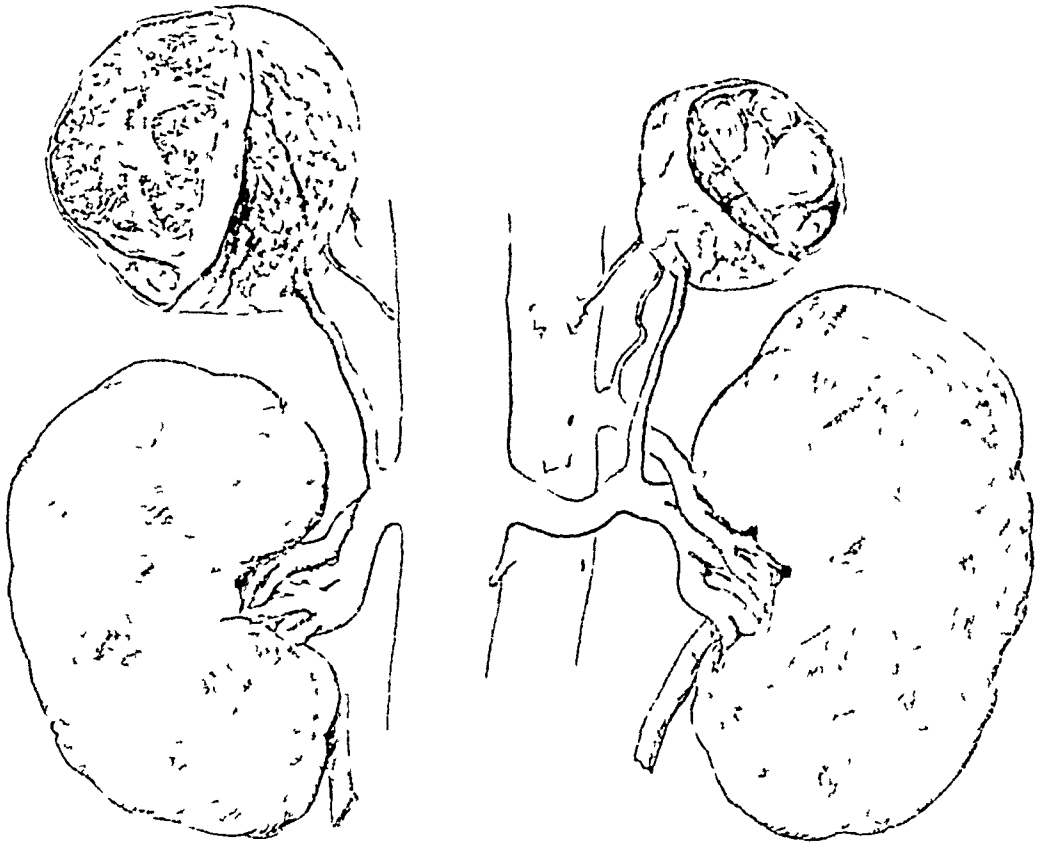


Fig 2—(Two thirds natural size)—Dog A Bilateral congenital cystic kidneys associated with bilateral primary adrenal hypernephromas The right adrenal tumor, with the exception of the subcapsular region, is almost entirely necrotic, with multiple regions of hemorrhage The left tumor is composed of whorls of tumor tissue, set in a connective tissue stroma, the large whorls are necrotic

of a carcinoma simplex, the solid masses here are composed of cells with a small round nucleus, quite granular with chromatin and deep staining with hematoxylin The cells are very indefinitely outlined, there being a few pink staining plasma shreds and granules between the nuclei In these softer regions the stroma is delicate with blood vessels running through it Still other regions of the tumor that are most fibrous to the gross examination are composed of almost solid hyalin connective tissue masses with single cells, chains and irregular groups of carcinoma cells scattered throughout

Lungs All the tumor nodules in the lungs are of thyroid tissue, as is the pedunculated nodule in the anterior mediastinum, and also a small nodule accidentally discovered in the region of the pineal body In the lungs the small nodules are in the blood vessels, some are necrotic, while others, especially the larger ones, have a fibrous and vascular stroma Most of the metastases tend to reproduce the same type of carcinoma tissue as was described in the thyroid tumor, some are adenocarcinomatous, while others are more of the carcinoma simplex type

Adrenals The microscopic picture is the same for both adrenal tumors, except that more necrosis is present in the sections taken from the tumor on the right side The capsule is thick and fibrous, yet very irregular in its thickness In either tumor the picture varies considerably in different places For

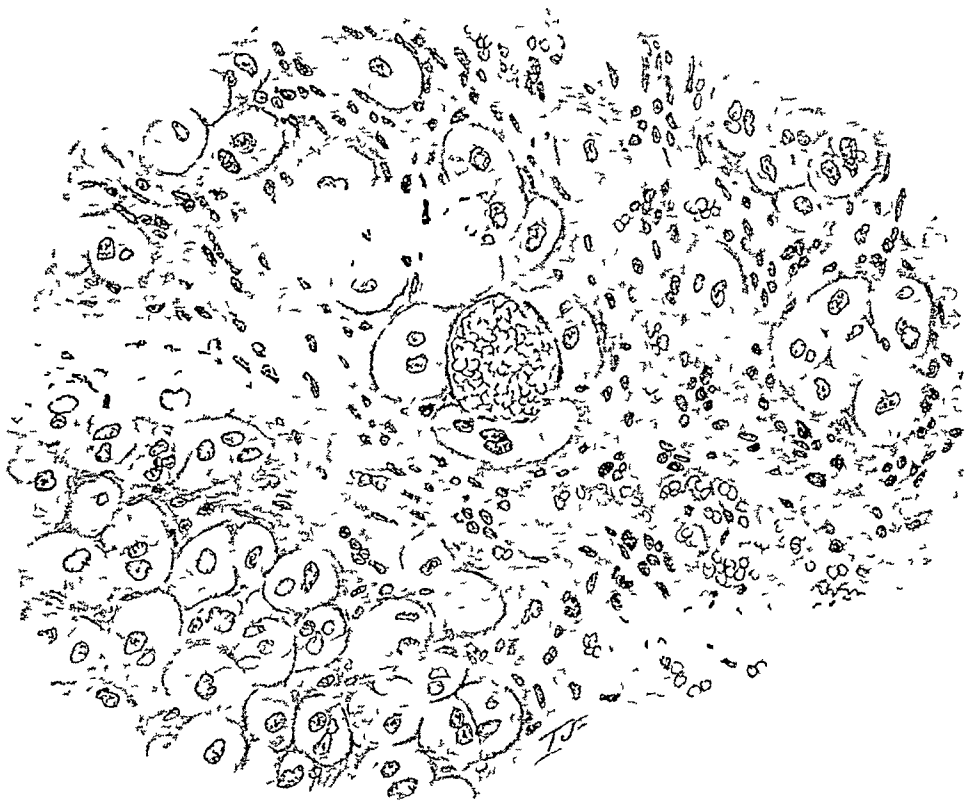


Fig 3—Dog A Central portion of adrenal tumor, represented by a large polygonal cell, having a distinct bladder like yet often solid nucleus, and a cytoplasm distinctly vacuoled These cells are arranged in single and in double cordons, in nests, or set singly in a hyaline connective tissue stroma

the most part, the central portion of both tumors is represented by a large polygonal cell, having a distinct bladder-like, yet often solid nucleus, and a cytoplasm distinctly outlined and vacuoled (Fig 3) These cells are quite similar to the ordinary foamy cells in hypernephromas of the kidney They are arranged in single and double cords, in nests, or set singly in a hyaline like connective tissue stroma Between the columns and cords are great numbers of blood sinuses, in fact, in places the dilated blood-sinuses have crowded and necrosed the tumor cells

In other places, smaller cells with a deeper staining nucleus and a less defined cytoplasm are found in large masses Between the cells a very delicate, embryonal connective tissue stroma is found This type of formation is mixed rather thoroughly in places with regions of the above described foamy cells

Just under the capsule in places is found an atypical zona fasciculata these cells sometimes become sarcomatous in appearance, approaching the spindle cell and the large round cell type

At the upper inner surface of the tumor on the right side, the cells under the capsule can be recognized as atypical cells of the zona glomerulosa Their cordons are very irregular being composed of flattened indistinctly outlined cells with deep staining irregular nuclei they are continuous with large thick solid bundles of oval cells the outer layer alone being recognized as cells from the glomerular zone

In the moon shaped cap described in the gross lying on the lower border of the right adrenal tumor there are masses of medullary or chromaffin cells, sharply angular with a large amount of eosin staining cytoplasm They are somewhat larger and more irregular than the normal medullary cells and in mass are separated sharply from the surrounding tumor tissue

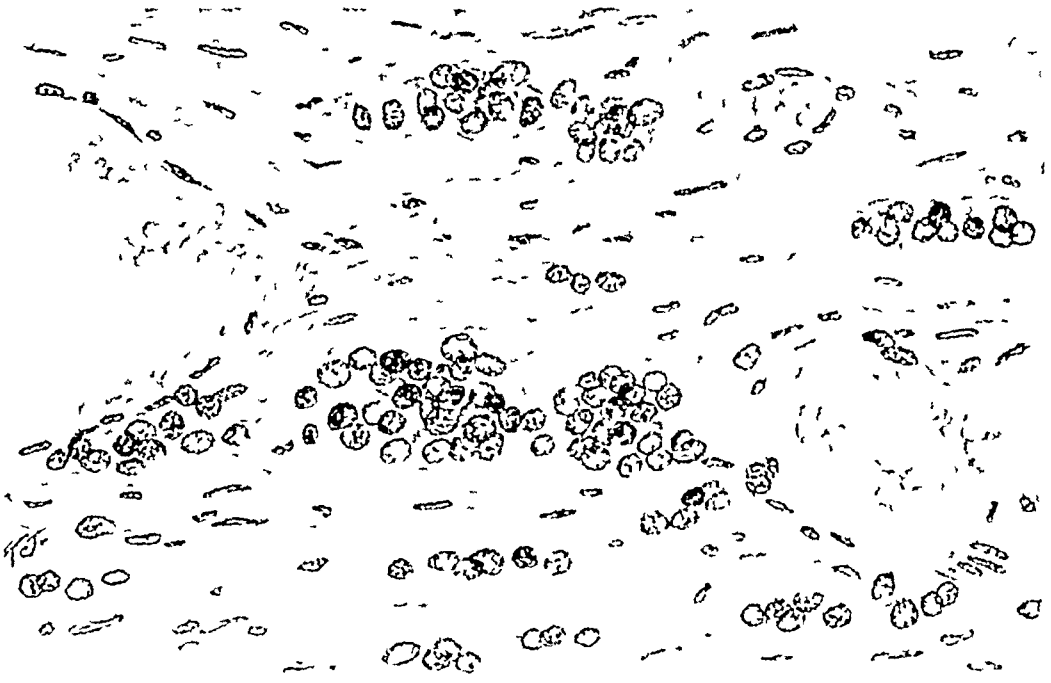


Fig 4—Dog A Capsule of adrenal tumor infiltrated by strings and small masses of tumor cells The cells are polygonal, with a small amount of dense purplish staining cytoplasm, and an irregular deep staining nucleus

The capsule of both the right and the left tumor is infiltrated by strings and small masses of tumor cells arranged quite like the strings of carcinoma cells at the edge of a carcinoma simplex (Fig 4) These cells are medium sized polygonal cells, with a purplish staining dense cytoplasm and an irregular deep-staining nucleus For the most part, these chains of cells do not penetrate except into the inner two thirds of the capsule In several places, however, they have broken through the capsule (Fig 5) and are found in irregular masses in the loose connective tissues on the outside Small bunches of them are found in large spaces, apparently lymph spaces in the connective tissue and areolar tissue outside the tumor Their appearance outside of the capsule is a little different from that within the interstices of the capsule, in that, outside, both the nucleus and the cytoplasmic outline of the cells are irregularly flattened The capsule is entirely broken through in places by masses of these cells In several sections are found irregular giant cells of the myeloid type, either singly or in small groups In no place throughout either tumor are there any tubule formations

like those described in about half the cases of renal hypernephromas Histologically, both tumors are essentially solid

There are veins on the surface of the right adrenal capsule that have been thrombosed, the lumen now being in the process of canalization A careful search for adrenal metastases was made in other parts of the body, but none were found

As a summary of the anatomical findings in Dog A, there are (1) adenocarcinoma of the thyroid with metastases in the lungs, mediastinum and pineal region, (2) bilateral primary adrenal mesothelioma, the local picture being that of early though definite malignancy, (3) bilateral congenital multicystic kidneys, and (4) cavernous hemangioma of the liver

CASE 2.—Dog B is a large old female The neck is diffusely enlarged, while in the posterior mammary region there is a nodular, rather soft mass from 4 to 6 cm in thickness, 8 cm wide and about 13 cm in length

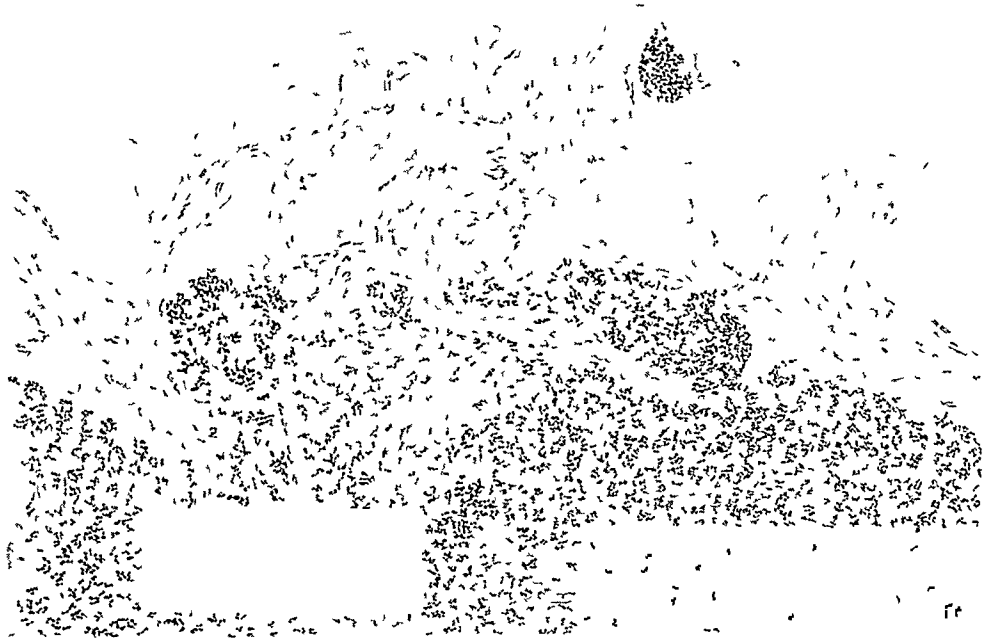


FIG 5.—Dog A Capsule of adrenal tumor under low power In several places the tumor cells have broken entirely through the capsule and are found in irregular masses in the loose connective tissue outside, and also in large spaces, apparently lymph-spaces

Thyroid The thyroid gland is quite like that of Dog A with the exception that it is not quite so fibrous and not so large There are a few lymph-nodes in the cervical region of both sides that contain white metastatic tumor nodules The tumor is very vascular, the vessels containing numerous papilliferous tumor thrombi

Lungs The lungs alone contain thyroid metastases, of which there are about fifty, ranging in size from 1 mm to 1 cm in diameter They are spherical and of a whitish gray color

Mammary Tumor The right posterior mamma is more involved than the left, though both are involved by the tumor The skin is immovable over the mass but is not ulcerated The tumor is roughly nodular, the nodules being from 2 to 5 cm in diameter, and indistinctly separated from one another by a softer internodular tissue On the surface made by sectioning the tumor it is seen that the border of the tumor is indefinite in its separation from the surrounding normal tissue, in places it has a very delicate capsule The region

between the nodules is milky white, and soft or gelatinous in consistency These nodules are nothing more than irregular firm cartilaginous centers surrounded by softer tissue The transition between the soft and the firmer tissues is a very gradual one

Adrenals The adrenals are twice as large as normal They are firm in consistency

The remaining tissues reveal no pathological changes that are of importance in this case

Microscopic The tumor of the thyroid is of the adenocarcinomatous variety tending toward the solidum or simplex type in this respect it differs from the carcinoma of the thyroid in Dog A The alveoli here are incompletely formed in most instances, and contain a granular eosin staining substance, more like albumin than like colloid



Fig 6—Dog B Mammary tumor The softer portions of this tumor are composed of a wildly proliferating sarcomatous like connective tissue, in which there are various-sized tubule formations some are solid while others have empty lumina, and are lined with cells from three to six layers deep, these cells do not resemble endothelial or epithelial cells but are of an intermediate type The above mentioned type of tissue runs into a myxomatous tissue, and this in turn into an embryonal cartilaginous tissue

The metastases in the cervical lymph nodes are similar to the original thyroid carcinoma tissue The nodules in the lungs are all metastases from the thyroid tumor They are located in the blood-vessels, the smaller ones consist of irregular cell nests tending to alveolar formation, in places, set in a hyaline like tissue, the larger nodules have a distinct fibrous stroma in which there are blood vessels The cells, though, in places tending to form alveoli are for the most part arranged in no particular manner

Mammary Tumor The edges of this tumor are faintly limited by a delicate connective tissue capsule which is, in places, lacking The outermost tumor-tissue, which is a diffusely proliferating sarcomatous like connective tissue, corresponds to the soft gelatinous internodular tissue described in the gross The latter has in many places no limiting connective tissue capsule between it

and the normal tissue The soft tissue is composed of irregular sized spindle-cells, the fibrillæ of which approach that of embryonal connective tissue in character The spindle cells are arranged in no regular fashion, but are confused and run in all directions (Fig 6) Within the tissue mentioned above lie many various sized, twisted and compressed tubule formations, the lumina of which are distorted and empty The cells lining these tubules are arranged in irregular stratified formation, from four to six cells deep They do not resemble endothelial cells or epithelial cells, but are of an intermediate type

In the centers of the tumor nodules there is invariably found a cartilaginous tissue which is not mature or typical in any place At its edges it fades into a myxomatous tissue, into a mucoid substance, or into the above described sarcomatous like connective tissue This microscopic picture is quite identical with that of the mixed tumors of the parotid gland described by Wilson and Willis[28]

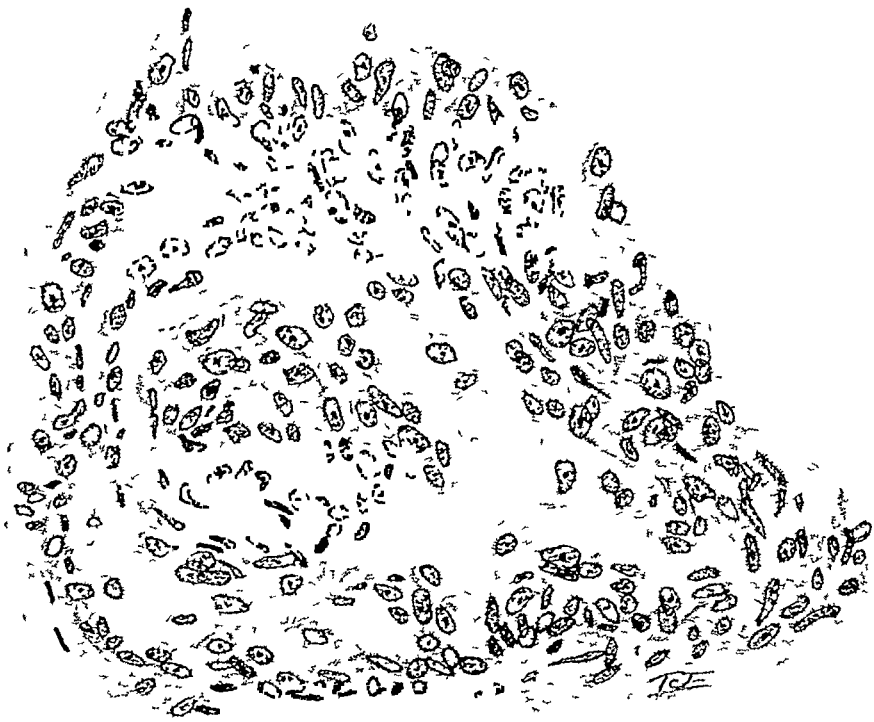


Fig 7 —Dog B Mammary tumor A high power drawing of an incompletely formed tubule, with its surrounding sarcomatous like connective tissue

There are no detectable metastases from this tumor, yet the histological character of the sarcomatous connective tissue on the advancing border of the tumor indicates probable malignancy The older portions in the centers of the nodules are composed of a tissue approaching the adult type The peripheral portions can be considered as a more embryonal and undifferentiated mesodermal tissue

Adrenals These, though grossly normal except for slight enlargement, are very interesting histologically, in that from fifteen to twenty one small intracapsular adenomas, composed of cells similar to the zona glomerulosa, are found in any one cross section

Briefly, then, in Dog B there are the following (1) adenocarcinoma of the thyroid gland with metastases in the lung, (2) mixed tumor of the mammary gland (chondrosarco endothelioma), and (3) multiple adenomas of the adrenal capsule

28 Wilson, L B, and Willis, B C Mixed Tumors of the Salivary Glands, Am Jour Med Sc, 1912, cxlii, 656

COMMENT

What inference may we draw from these two cases? Is there any one etiological factor responsible for the development of these multiple primary malignant tumors in either case, or are they incidentally multiple?

It is well known that malignant disease of the thyroid gland in man and in the dog is more prevalent in goitrous districts than in non-goitrous districts In Great Britain, where goiter is not prevalent, only seven cases of malignant disease of the thyroid were found in 7,294 necropsies[29] In Vienna, where goiter is not uncommon, Hinterstoisser[30] found fifty malignant tumors of the thyroid in 18,117 necropsies On the other hand, in Berne, where goiter is common, Limacher[31] found eighty-two cases of malignant disease of the thyroid in only 7,611 necropsies, thirty-eight of these were carcinoma, while forty-four were sarcomas Marine and Lenhart[32] have shown from their work on brook-trout that the goiters of fish are entirely due to metabolic disturbances from food and external conditions They[33] state the following in reference to malignant disease of the thyroid gland "Goiter in some way favors the development of cancer, just as it favors the development of benign tumors Cancer developing in an otherwise normal gland is extremely rare We have never observed thyroid cancer apart from pre-existing goiter"

The ordinary goiter in dogs is due usually to a diffuse enlargement of the gland, and rarely to adenomas, Marine and Lenhart assert that fetal and that simple adenomas are never found in dogs In and about Chicago, where goiters in dogs are very common, carcinoma of this gland is not at all infrequently found

In both Dogs A and B there is nothing that indicates a preexisting developmental anomaly, presumably the thyroid carcinomas in both cases were preceded by, and indirectly due to, a diffuse hyperplasia, which in turn is no doubt, so far as is known, a manifestation of metabolic disturbance dependent on the water and food

In Dog A, in addition to the thyroid carcinoma, there are the bilateral adrenal hypernephromas, or better, "mesotheliomas," so far as it has

29 Muller, G P, and Speese, J Malignant Disease of the Thyroid, Univ Penn Med Bull, 1906, xix, 74

30 Hinterstoisser, H Beitrage zur Lehre vom Schilddrüsenkrebs, Beitr z Chir Festschrift Billroth, 1892, p 287

31 Limacher Quoted by Muller and Speese (See Footnote 29)

32 Marine, D, and Lenhart, C H Observations and Experiments on the So Called Thyroid Carcinoma of Brook-Trout and Its Relation to Ordinary Goiter, Jour Exper Med, 1910, xii, 311

33 Marine, D, and Lenhart, C H Pathological Anatomy of the Human Thyroid Gland, The Archives Int Med, 1911, vii, 506

been rather conclusively shown that renal hypernephromas and adrenal hypernephromas are distinct from each other both embryologically and histologically These two adrenal tumors have undoubtedly originated from an anomalous embryonal development, since they are bilateral, and are associated with bilateral, congenital, multicystic kidneys The cavernous hemangioma of the liver is of doubtful embryonal development There is, then, nothing in common in the etiology of the thyroid carcinoma and of the double adrenal mesothelioma, one being of probable indirect metabolic origin, while the others are from embryonal anlages Their location does not suggest that they are the result of any one tissue susceptibility, the adrenals being of mesothelial origin, while the thyroid is of ectodermal origin

In Dog B, besides the carcinoma of the thyroid gland, there is the mixed tumor of the mammary gland, similar in structure to those mixed tumors in the parotid gland This tumor, like the adrenal tumors in Dog A, without much doubt is of embryonal origin It is closely allied to the teratomas, having cartilage, myxomatous tissue, endothelial structures and a sarcomatous stroma The various grades of teratoma and mixed tumors are most frequently found in the ovaries, testes, parotid gland and mammary gland In Dog B the occurrence of multiple capsular adenomas of the adrenals is significant of an imperfect embryonal development

Both the malignant tumors in Dog B, then, also are, as in Dog A, very probably of different etiology, the one being due indirectly to metabolic disturbance, and the other, the mixed tumor, being in some way connected with an embryonal anlage One is of ectodermal and the other of mesodermal origin

SUMMARY

Multiple primary malignant tumors occur in approximately 0 2 per cent of all cases of malignant tumors

Certain of them that originate close together in one type of tissue are indicative of a localized tendency to malignant degeneration

Others that are essentially bilateral, occurring in the ovaries, testicles, kidneys and adrenals, are indicative of a tissue predisposition, dependent in most instances on an anomalous embryonal development

Besides these two classes there is another general class of cases, two-thirds of which is composed of multiple primary malignant tumors, the location and arrangement of which is strongly indicative of a system involvement, affecting mainly the reproductive, the gastro-intestinal and the cutaneous systems

In the other third of this class of cases, the tumors are distributed miscellaneously, and apparently have no common features The two

cases here reported belong to the latter class, each animal having one tumor dependent indirectly on a metabolic disturbance, and another tumor distinctly of embryonal anlage

I wish to thank Dr D J Davis for his kind suggestions in the compilation of this paper

1532 Congress Street

FATTY INFILTRATION (LIPOMATOSIS) OF THE AURICULOVENTRICULAR BUNDLE OF HIS, WITH SUDDEN UNEXPECTED DEATH

FRANK NUZUM, M D
CHICAGO

Numerous studies have established a definite relationship between the symptoms usually referred to as Adams-Stokes disease and changes in the auriculoventricular bundle of the heart, the bundle of His The absence of sufficient information in many of the reports in a recent summary by Bachman[1] led him to base this definite relationship on the evidence in only twenty-four of the eighty-six cases reviewed, in the remainder the details were inadequate for comparison

In these twenty-four, to which I have added a few[2] not in the number considered by Bachman, the alterations found in the His bundle were as follows

Condition	Cases
Gumma	7
Fibrosis	7
Calcified plaques	3
Lipomatosis	2
Round celled infiltration	3
Arteriosclerosis of the bundle vessels	2
Tumor	1
Anemic infarct	1
Parenchymatous degeneration	1

From a study of such a table as this it is evident that well-marked examples of the Adams-Stokes syndrome have for their explanation a lesion of the auriculoventricular bundle A number of careful studies have shown that a complete transverse lesion of the main stem of this conducting system is followed by a total heart-block

It is possible, however, that when the auriculoventricular bundle possesses alterations only a portion of the symptoms regarded as essential for the Adams-Stokes syndrome are manifested Beginning or partial involvement of the bundle may lead simply to a lengthening of the inter-

* Submitted for publication Dec 5, 1913

* From the Pathological Laboratory of the Rush Medical College

1 Bachman A Physiologico Pathological Study of a Case of Heart Block Occurring in a Dog as a Result of Natural Causes, Jour Exper Med, 1912, xvi, 3

2 Keith and Miller Lancet, London, 1906, ii, 1429, Heinecke Drei Falle von Adams Stokes Krankheit, Berl klin Wchnschr 1907, xliv, 1125, Karscher and Schaffner Ein Fall von Adams Stokes 'scher Krankheit mit Schwiele im His 'schen Bundle, Berl Klin Wchnschr, 1908, xlv, 1266

systolic period[3] Or there may be only bradycardia tachycardia, arhythmia or cyanosis[4] Furthermore, in a few instances sudden death has occurred without, so far as known, any illness with symptoms leading to even a suspicion of Adams-Stokes disease Moenckeberg[5] has reported two such occurrences

The first was diphtheria with sudden death Moenckeberg does not state at what period in the disease death occurred No gross changes were found in the myocardium at the post-mortem examination The auriculoventricular bundle and its many branches when sectioned serially in paraffin and stained by Van Gieson and hematoxylin appeared normal Using Scharlach R, however, the main stem both branches and their subdivisions were found to be the site of a distinct fatty change which sharply differentiated this system from the fat-free myocardium

In Moenckeberg's second example sudden death followed trephining of the skull, as a result of caries of the frontal bone The myocardium presented fatty changes In Scharlach R preparations both divisions of the His bundle stood out in marked contrast due to the greater fatty change that was present in them Yet there were individual fibers among these unaffected and normal in all respects

Acute fatty changes similar to these and caused by septicemia pneumonia, typhoid fever, diffuse bronchitis and chloroform poisoning have been studied by Sapegno[6] Among other results of the studies of seventy-two hearts he concluded that rapid, unexpected death of an individual in whom no other anatomic alteration is found may be due to an acute or a chronic lesion of these peculiar fibers in the heart He also believes that while fatty changes are often present in both the myocardium and the bundle system together, yet in 50 per cent of all instances these acute changes affect especially the conducting system

Similar alterations have also been found by Aschoff[7] in the His bundle He has not however, observed any instance in which such a change was responsible for the sudden death

Marked fatty changes of an *entirely different* sort than those discussed by Moenckeberg, Aschoff and by Sapengo as quoted above, may be very

3 Erlanger On the Physiology of Heart Block in Mammals with Especial Reference to Causation of Adams Stokes Disease Jour Exper Med 1906 viii, 8

4 Von Neusser Disorders of the Respiration and Circulation, 1908 (New York), Part 2, p 18

5 Moenckeberg Untersuchungen uber das auriculoventriculare Bundle bei Mann, Jena, 1906, Cases 71 and 104, p 318

6 Sapegno Nuove Ricerche sulla Patologia del Fascio atrioventricolare, Arch per le Sc med, 1910, xxxiv, 549

7 Referat uber die Herzstorungen in ihrem Beziehungen zu den Spezifischen Muskelsystem des Herzens, Verhandl d Deutschen patholog Gesellsch, 1910, xiv, 3

extensive in the His bundle, as is well shown by the following report of fatty infiltration or replacement of the His bundle by adipose tissue associated with sudden death

History.—A man 38 years of age, who had always been well, died suddenly. Friends stated that at times he had seizures during which he trembled generally and voluntarily or consciously shook his hands and forearms rapidly. These only lasted a moment or two and there was no loss of consciousness. So far as is known he never sought medical advice.

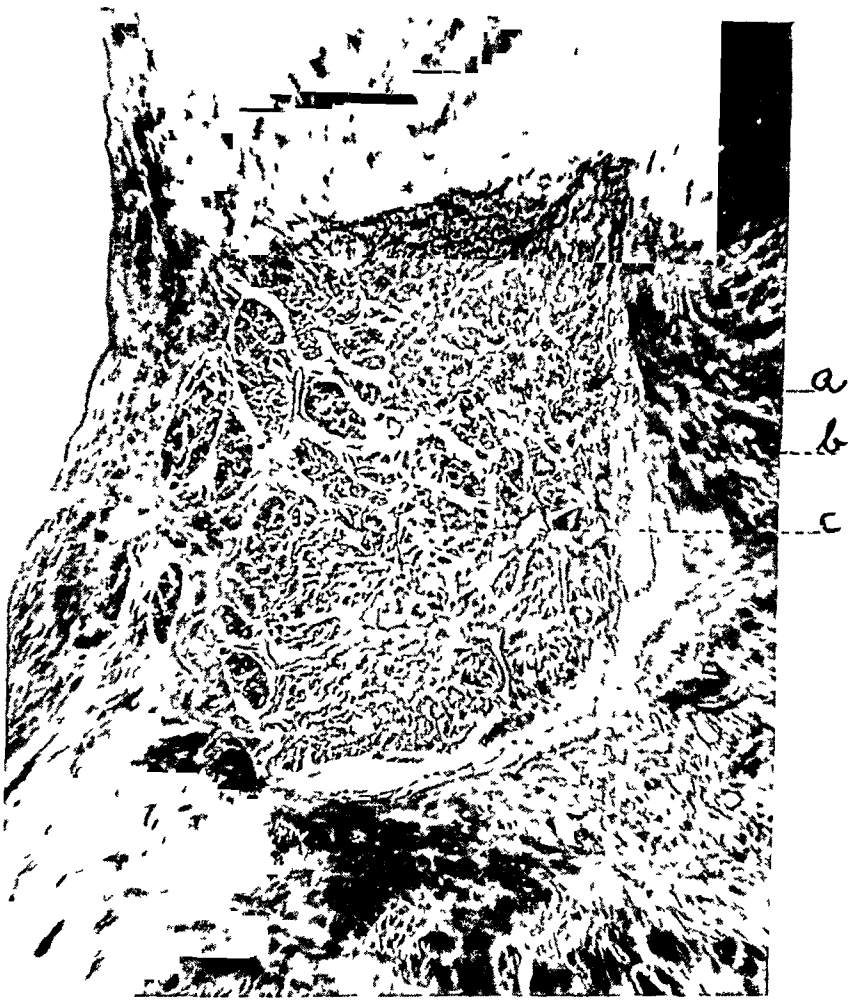


Fig. 1.—A transverse section through the distal portion of the normal node of the His bundle at a point in its passage through the auriculo-ventricular fibrous ring. a, Bundle fibers; b, Fibrous connective tissue surrounding the bundle; c, Bundle vessels.

Necropsy.—The body was that of a powerful, well nourished white man with no external injuries. There was no excess of subcutaneous adipose tissue. The organs were found to be of normal size and position. There were no gross evidences of disease anywhere. Careful examination of the gastro-intestinal tract failed to disclose any alterations leading to a suspicion that death might have resulted from poisoning, nor did the circumstances attending his death indicate this possibility.

The heart was not hypertrophied and in so far as could be ascertained by gross examination was normal in all respects save for a slight degree of coronary sclerosis and a moderate fibrous myocarditis There was no abnormal deposit of fat on the outside of the heart

"There are approximately two small nodules in each coronary artery The first nodule in each vessel is 1 5 cm distal to the sinus of Valsalva The nodules involve the intima of the vessel wall chiefly and the lumen is but slightly lessened in each instance On tracing the coronary arteries to their terminal endings no occlusions are found The vessels are not tortuous and the walls are elastic"



Fig 2—Adipose tissue in the node a Central fibrous body surrounding the node b Intact bundle fibers c Adipose tissue d Bundle vessels

Portions of both the right and left ventricular walls, the septum and papillary muscles were examined at various levels to determine the amount of fibrosis

"The septum contains only a normal amount of fibrous connective tissue The fibrous tissue in the ventricular walls is somewhat increased in amount At all points this increase is only slight and but few true muscle fibers are replaced This change is not especially prominent in any portion of either ventricular wall The amount of fibrosis in the papillary muscles is even less than that observed in the outer ventricular wall"[8]

8 Dr E R LeCount, who conducted the post-mortem examination, was of the opinion that the gross changes in the heart were inadequate to explain death

The following anatomic diagnosis was made

"Slight nodular sclerosis of the coronary arteries, slight fibrous myocarditis, slight fibrous mural endocarditis, passive hyperemia of the liver, spleen, kidneys, pancreas and small bowel, petechial hemorrhages in the large and small bowel and in the pleura of the right lung, marked chylangiectasis of the vessels of the small bowel—post mortem (?), hyperplasia of the tracheobronchial lymph nodes, of the mesenteric lymph glands, of the spleen, of the solitary follicles of the small bowel and of the lymphoid tissue of the pharynx, marked emphysema of both lungs, edema of the pharyngeal mucous membrane, caseous infratracheal lymph nodes, fibrous patch in the pia of the left frontal lobe of the brain, slight sclerosis of the aorta and of the vessels at the base of the brain, fibrous adhesions between

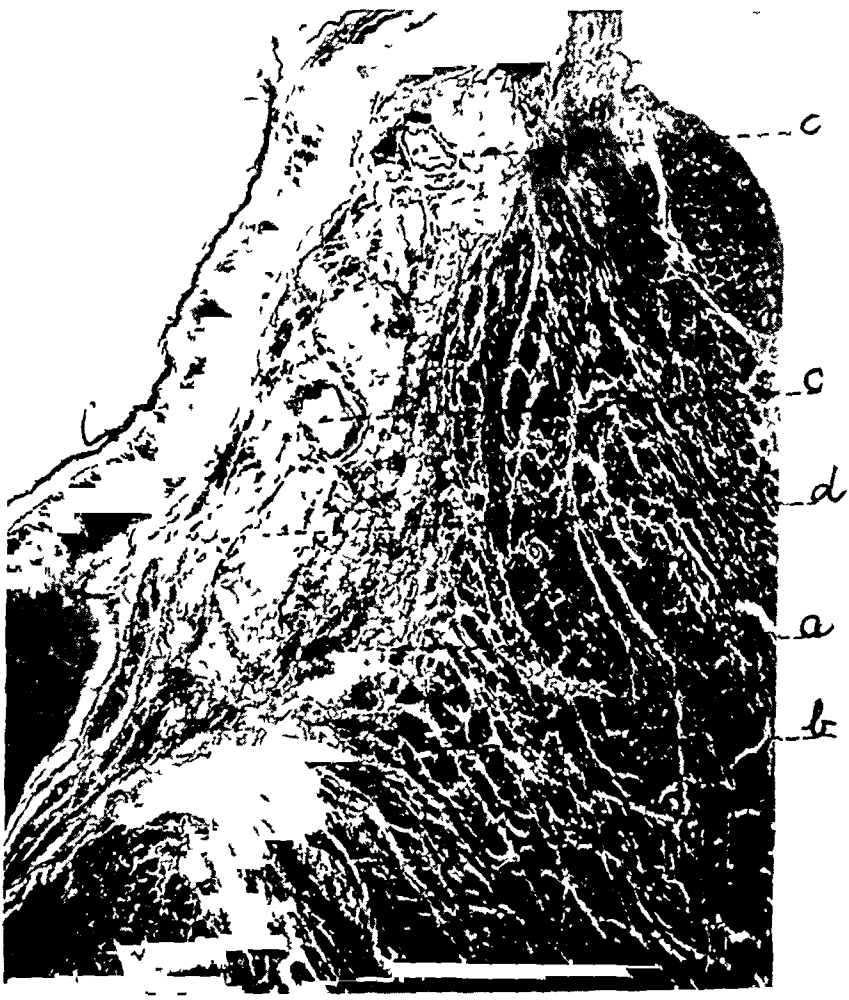


Fig 3.—Section distal to that shown in Fig 2 Two thirds to three fourths of the bundle fibers are replaced by adipose tissue in this and adjacent sections a Intact bundle fibers b Beginning of left ramus c Bundle vessels d Adipose tissue

the gall bladder and the first portion of the duodenum, fibrous pleuritis small fibrous patches in the anterior mitral leaflet, slight fibrous subepicardial and supra arterial nodular pericarditis, marked right hallux valgus, carious and missing teeth"

Because the gross alterations were so few a study of the His bundle was undertaken Histological examination was carried out by means of serial paraffin sections of the entire bundle down to and for a considerable distance beyond the

point of origin of the right and left branches Further study was made of the terminal extensions at various levels in the ventricular walls Every fifth section was mounted and various stains employed

In these sections there is a very marked fatty infiltration limited strictly to the auriculoventricular bundle and its branches, the myocardium containing no adipose tissue This change is moderate in the nodal region, more marked in the main stem At the point of origin of the right and left branches nearly the entire bundle is absent and in its place there is adipose tissue Both of these branches and the Purkinje fibers at whatever plane examined present such alterations in varying degree

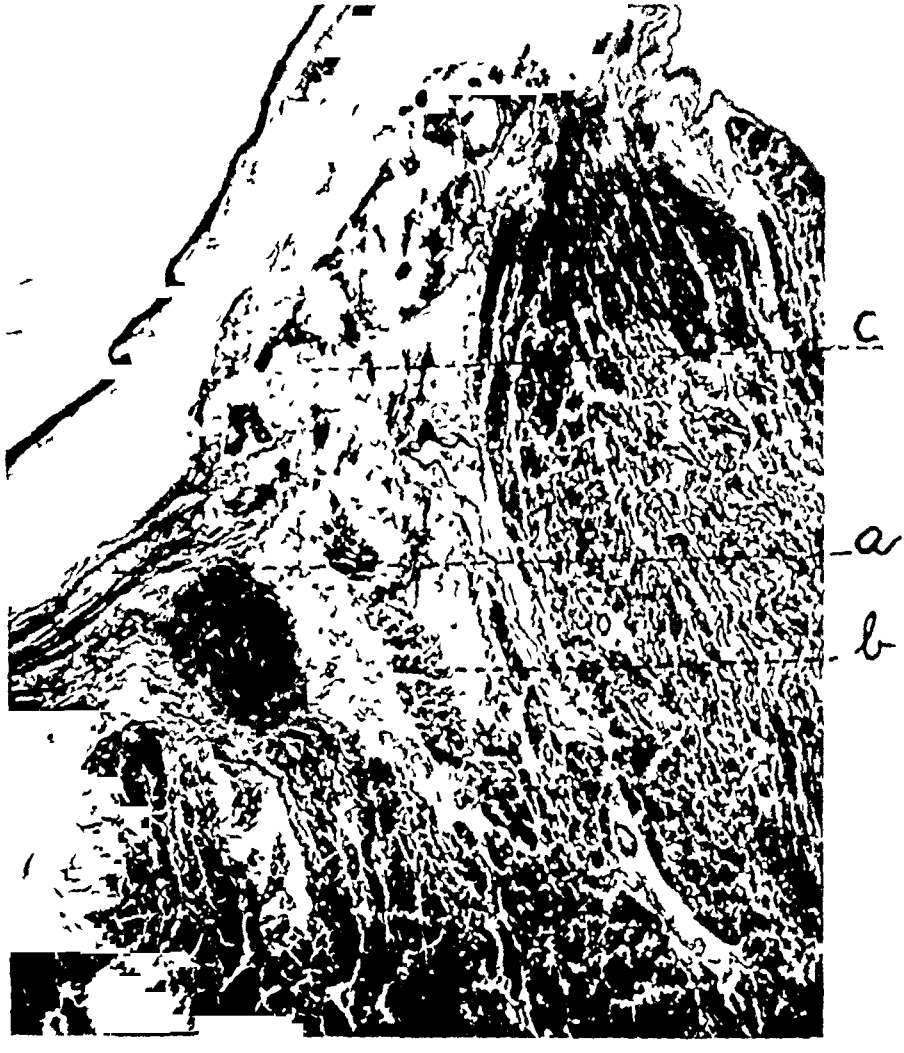


Fig 4 —Section distal to that shown in Fig 3 A portion of the left ramus and the beginning of the right ramus are present here In this section 85 to 95 per cent of the bundle fibers are replaced by adipose tissue a Left branch of bundle b Beginning of right ramus c Main portion of bundle

Both the muscle fibers and the connective tissue of the bundle system are replaced by adipose tissue If that part of the bundle lying between the proximal part of the node and the point of division of the main stem into right and left branches is arbitrarily divided into three segments then approximately 20 per cent (Fig 2) of the fibers and connective tissue framework of the first segment of the bundle is replaced by adipose tissue, 65 to 75 per cent (Fig 3) of the middle segment and 85 to 90 per cent (Fig 4), of the distal segment is similarly

affected Just distal to the bifurcation of the bundle 50 per cent of the structure of the right branch is replaced by adipose tissue while at a similar location in the left branch only 10 to 15 per cent of the fibers are so affected These alterations are present, as before stated, in the terminal portions of this specific system of muscle fibers throughout their subendocardial course The adipose tissue, limited sharply to the Purkinje fibers, differentiates this system at a glance from the surrounding myocardium In fact their location is readily determined with a hand lens or the low power of a microscope and followed from section to section by the adipose tissue fat holes at such points

Some of the muscle cells of the Purkinje fibers are normal Others on cross section show fat globules in their centers These globules vary in size from small droplets to larger drops which replace a portion of the protoplasm, leaving the



Fig 5.—Terminal "specific" muscle fibers sectioned across, phosphotungstic acid hematoxylin stain a Typical arrangement of fibrillae about the cell periphery Protoplasm in center of cell replaced by fat b Fibrillae dividing cell into fields Center of fields occupied by fat c Entire cell almost entirely replaced by adipose tissue

fibrillae standing out very sharply about the periphery of the cell (Fig 5a) Again, the fibrillae may divide the cell into small fields, the center of these fields being occupied by fat droplets (Fig 5b) Or the muscle cell may be so distended with fat globules that the remaining fibrillae have all been crowded into one corner of the cell many of them absent Finally the entire muscle fiber with its fibrillae, cell membrane and surrounding sheath of connective tissue may be entirely absent and its place taken by adipose tissue

As a rule, this replacement by adipose tissue, lipomatosis, which is quite regularly found in the auriculoventricular bundle system of elderly people is not extensive enough to lead to clinical manifestations, yet Fahr[9] has reported so marked a lipomatosis of the bundle that the Adams-Stokes complex resulted and finally death Aschoff and Tawara[10] have made a similar observation, but Sapegno[11] states in his second article that they later denied the occurrence of a true Adams-Stokes symptom-complex in this instance

Sapegno[11] describes a lipomatosis found in the His bundle in the heart of a young girl who died twelve days after recovery from typhoid fever He is very positive in his statements that death in this instance, which was sudden, was not due to the acute fatty changes in the myocardium which are sometimes observed after typhoid fever Furthermore, this young girl had a cardiac insufficiency which was present from birth and the lipomatosis of the bundle which he found he regarded as congenital On account of the absence of other gross alterations or processes of disease in the body, death was attributed to the presence of the very marked lipomatosis of "from the point of division of the main branch into two rami to a point where these again decrease rapidly in size"[12]

He concurs with Monckeberg in recognizing that there is a tendency toward adiposity of the auriculoventricular system in old age, and that while, as a rule, this adiposity is accompanied by fatty infiltration of the myocardium generally, that the bundle alone may be affected and possess grossly visible fat

In conclusion, without attempting to predicate that death in this instance was necessarily due to these alterations in the His bundle, it is believed that such an examination as this in instances of sudden death with symptoms of cardiac disease, where there are relatively few gross changes in the heart, should be more frequently made As has been suggested by other investigators of conditions in the auriculoventricular bundle, the nerves connected with it should also be examined

9 Fahr Cited by Monckeberg Berl klin Wchnschr, 1909, xlvi, Part 2, p 45

10 Aschoff and Tawara Die Pathologische anatomische Grundlagen der Herzschwache, Fisher, Jena, 1906

11 Sapegno Ricerche sulla patologia del fascia atrio ventricolare, Arch per le sc med, 1910, xxxiv, 143

12 The original is as follows "Il cusicinetto adiposo si mantiene sempre di una massa notecole fino alla divisione del f nei suoi due rami, al qual livello diminuisce rapidamente di volume e cessa quasi del tutto"

BLOOD-PRESSURES

THEIR RELATION TO EACH OTHER AND TO PHYSICAL EFFICIENCY *

JOSEPH H BARACH, M D, AND W L MARKS, M D
PITTSBURGH, PA

In this communication we desire to record the blood-pressure findings in a total of 742 males with particular reference to the diastolic and pulse pressures, also the relations between blood-pressure and physical efficiency

METHODS

In these blood-pressure readings we used a mercury manometer of the usual type with 12 cm cuff, and we made our determinations by the auscultatory method

The maximum pressure was determined in all cases at the first sound The minimum pressure in Series A was read at the time of disappearance of all sounds (fifth phase), and in Series B, more accurately at the point of the last clear sound preceding the final dull tones This is known as the fourth phase Obviously, then, we get a reading somewhat higher when we make our readings at the final sharp tone than if we wait for the disappearance of all sounds The difference may be as much as 10 mm and usually in the neighborhood of 5 mm This difference, while noteworthy, is not sufficiently great to detract from the actual value of our figures for the minimum blood-pressure in Series A in the manner in which we use them

The subjects in this study were students in their usual health, most of them between 15 and 25 years of age While our records include the readings in both the erect and horizontal posture, the accompanying tables and figures refer only to the readings in the erect, the effect of change of posture on the arterial and venous pressures having been previously studied by us [1]

The method used in estimating the physical efficiency in this work is the one known as the "intercollegiate standard" It consists of adding the strength of back plus strength of legs, plus strength of right and left forearms, plus lung capacity divided by twenty, plus body-weight divided

* *Submitted for publication Jan 6, 1914*

* From the Department of Student Health, Carnegie Institute of Technology

1 Barach, J H, and Marks, W L Effect of Change of Posture without Active Muscular Exertion on the Arterial and Venous Pressures, ARCHIVES INT. MED, 1913, xi, 485

by ten The last figure obtained is multiplied by the number of times the subject pulls up and dips his body by the strength of his arms and shoulder muscles This gives us a very fair estimate of the individual's physical condition For one to show a high total strength he must possess a good musculature, good coordination, an efficient circulatory and respiratory system, one which will respond properly and maintain itself during the effort required by the test

MINIMUM PRESSURES

In the accompanying tables it will be noted that we do not give the average height of the blood-pressure readings of an entire series of cases, but rather do we give the actual number of cases which occurred at the various heights We believe this to be more accurate and leading to truer conclusions

TABLE 1—AGE DISTRIBUTION IN A SERIES OF 552 CONSECUTIVE CASES

Age, Years	Cases
15 to 17	27
17 to 19	200
19 to 21	202
21 to 23	71
23 to 25	31
25 to 27	12
27 to 29	6
29 to 31	1
Over 31	2
Total	552

Maximum Pressure—As heretofore stated, the maximum pressure was read at the first sound—by the auscultatory method In 656 cases the distribution was as shown in Table 2

TABLE 2—DISTRIBUTION OF MAXIMUM PRESSURE IN 656 CASES

Mm Hg	Cases
90 100	7
100 110	23
110 120	112
120 130	204
130-140	153
140 150	95
150 160	45
160 170	14
170 180 180-190 190-200	3
Total	656

Out of these 656 cases, there are 594 under 150 mm Hg , these comprise 90 per cent of the entire series It has been our experience that just about 10 per cent of the men come in presenting a blood-pressure considerably higher than normal at their first examination, but at a

subsequent examination it is found to be much nearer the normal level This undoubtedly is due to the psychic effect of the examination

Minimum Pressure, Series A—Here the pressure was read by the auscultatory method at the disappearance of all sounds, with the results shown in Table 3

TABLE 3—MINIMUM PRESSURE READ BY THE AUSCULTATORY METHOD AT DISAPPEARANCE OF SOUNDS

Mm Hg	Cases	
30- 40	1	
40 50	2	
50 60	4	
60 70	20	327 Cases = 96 %
70- 80	82	
80 90	140	
90 100	78	
100 110	9	
110 120	1	
120 130	1	
Total	338	

The majority of these, 96 per cent, had a minimum pressure under 100 mm Hg

Minimum Pressure, Series B—In these cases the point of the minimum pressure was read at the last clear sound preceding the final dull tones (Table 4) It is now generally agreed that this is the exact point of the minimum pressure

TABLE 4—MINIMUM PRESSURE READ AT THE LAST CLEAR SOUND

Mm Hg	Cases	
40 50	1	
50- 60	4	
60- 70	12	273 cases or 87 %
70 80	41	
80- 90	94	
90 100	121	
100-110	27	
110-120	9	
120 130	3	
Total	312	

In this series, in which the point of minimum pressure is read earlier than in Series A, we find that over 87 per cent of the cases present a minimum pressure under 100 mm Hg, and that 256 cases, or 82 per cent, occur between the points of 70 mm and 100 mm Hg

We see, then, that when the minimum pressure is read at the fourth phase, 87 per cent of the cases are found to be not over 100 mm Hg, while at the end of the fifth phase 96 per cent of the cases do not exceed 100 mm Hg

In Series B, in which the pressure was read at the fourth phase, the 13 per cent which had a maximum pressure of over 100 mm were reexam-

ined We then found that the first was only a temporary response to the psychic effect of the examination and that in only two cases did the minimum blood-pressure exceed 100 mm, one showing a minimum blood-pressure of 105 and the other 102 mm Hg

Minimum Pressure in All Cases—If we allow for clinical purposes that the difference between the end of the fourth and fifth phase in a large series of cases such as this one is a negligible factor, by combining Series A and Series B we get the results shown in Table 5.

TABLE 5—MINIMUM PRESSURE IN ALL CASES

Mm Hg	Cases	
30 40	1	600 cases or 92 % Not over 100 mm Hg
40 50	3	
50- 60	8	
60- 70	32	
70- 80	123	
80 90	234	
90 100	199	
100 110	36	
110 120	10	
120 130	4	
130 140		
Total	650	

Pulse-Pressures—In Series A and B we have pulse-pressure readings in 629 cases The normal variation in the size of the pulse-pressure is seen to be quite marked

TABLE 6—PULSE-PRESSURES IN SERIES A MINIMUM PRESSURE READ AT THE FIFTH PHASE

Mm Hg	Cases
10 20	9
20 30	30
30 40	68
40 50	76
50 60	62
60 70	43
70 80	17
80 90	7
Total	312

TABLE 7—PULSE-PRESSURE IN SERIES B MINIMUM PRESSURE READ AT THE FOURTH PHASE

Mm Hg	Cases
5- 10	1
10 20	11
20- 30	45
30- 40	79
40 50	79
50- 60	53
60- 70	25
70 80	18
80 90	5
90 100	1
Total	317

Tables 6, 7 and 8 show clearly that we may hold the usual pulse-pressure as ranging between 20 and 70 mm Hg, in healthy persons of the ages here referred to

TABLE 8.—PULSE PRESSURE IN SERIES A AND B COMBINED

5- 10	1	
10- 20	20	
20- 30	75	560 cases or 88% Between 20 and 70 mm Hg
30- 40	147	
40- 50	155	
50- 60	115	
60- 70	68	
70- 80	35	
80- 90	12	
90-100	1	
Total	629	

RELATION BETWEEN THE PULSE-PRESSURE AND DIASTOLIC PRESSURE

Strassburger[2] attempted to find a blood-pressure quotient by dividing the pulse-pressure by the maximum pressure, but that has been shown to be erroneous It has recently been stated by Stone[3] that the pulse-pressure in the normal individual is equal to 50 per cent of the diastolic pressure, and that for clinical purposes we may assume that, when the pulse-pressure is over 50 per cent of the diastolic pressure, we are dealing with cardiac overload If this theory could have been proved, it would have been an important advance in our clinical work

We set forth here the findings in 742 cases Series A has its slight error on account of the minimum pressure having been read at the fifth phase, while in Series B this element is eliminated The figures represent the percentage relation of pulse-pressure to diastolic pressure Our formula is D P P P 100 ×

TABLE 9.—SERIES A MINIMUM PRESSURE READ AT FIFTH PHASE

Per Cent	Cases			
10- 20	10			
20- 30	24			
30- 40	48			
40- 50	49	108 cases, 30%	201 cases, 60%	264 cases, 70%
50- 60	59			
60- 70	45			
70- 80	39			
80- 90	23			
90-100	14			
100-110	16			
110-120	2			
120-130	1			
130-140	5			
Total	335			

2 Strassburger Arch f klin med, 1905, lxxxv

3 Stone, Willard, J The Clinical Significance of High and Low Pulse Pressures with Special Reference to Cardiac Load and Overload, Jour Am Med Assn, Oct 4, 1913, p 1256

TABLE 10.—SERIES B MINIMUM PRESSURE READ AT THE FOURTH PHASE

Per Cent	Cases			
5 10	2			
10- 20	8			
20 30	44			
30 40	63			
40 50	73	141 cases, 34%	260 cases, 63%	333 cases, 81%
50 60	68			
60 70	56			
70 80	29			
80 90	32			
90-100	11			
100 110	13			
110 120	1			
120 130	2			
130-170	5			
Total	407			

In Series B, in which the pulse-pressure is smaller than in Series A, we note that a greater number of cases occur in the lower percentages In both A and B we may note that the greatest number of cases do occur near the 50 per cent mark, and if we were to strike an average it would be close to 50 per cent , yet this would be far from representing the actual truth These tables show that there is a wide range both above and below the 50 per cent mark in normal individuals Our findings here would warrant only a statement that the pulse-pressure equals from 20 to 80 per cent of the diastolic pressure in about 75 per cent of the cases One objection may be offered to our deductions, and that is the comparative youth of the subjects Considering the age, the physical development, the height of the blood-pressures, and in the light of our previous observations on older subjects, we incline to believe this objection a negligible factor, and that our findings here are representative for normal persons up to middle life Other observations on older individuals in health and in various disease conditions with which we expect to deal in another paper, will bear out this point

RELATION OF TOTAL STRENGTH TO BLOOD-PRESSURES

We have pointed out previously[4] that there is no constant relation between blood-pressures and total strength In Table 11 we show that there is no definite relation between the pulse-pressure per centage of diastolic pressure and circulatory efficiency, such as is indicated by the general physical efficiency

The upper 146 cases give a pulse-pressure equal to 56 per cent diastolic pressure, while the lower 150 cases (excluding the last three) give

4 Barach, Joseph H Physiological and Pathological Effect of Severe Exertion (the Marathon Race) on the Circulatory and Renal System, THE ARCHIVES INT MED , 1910, v, 382

a pulse pressure equal to 61 per cent of diastolic pressure, showing that there is no relation between the total strength and pulse-pressure per cent of diastolic pressure

It is particularly worthy of note that the man who showed the highest total strength (1724), had a pulse-pressure equal to 100 per cent of the diastolic pressure This man certainly had no evidence of cardiac overload Also that one subject whose strength was only 651 (the fourth lowest of the 344 cases), showed a pulse-pressure equal to 80 per cent of the diastolic pressure

TABLE 11.—The Relation of Total Strength to Blood Pressures

Total Strength	Cases	Av Str	Average Max P	Average Min P	Average P P	Average P P %
400 500	1	477	117	75	42	56
400 500	2	445	134	82	50	62
600 700	1	651	126	70	56	80
600- 700	5	860	129	66	44	54
600- 700	3	647	121	80	41	54
700 800	5	774	129	88	41	47
800 900	5	860	129	66	44	54
800 900	10	865	123	75	47	64
200 900	35	871	126	84	41	51
900 1,000	3	927	130	83	46	59
900 1,000	19	950	126	81	42	53
1,000 1,100	28	1,045	121	78	42	51
1,000 1,100	4	1,093	117	83	33	45
1,000 1,100	25	1,048	138	67	51	66
1,100 1,200	14	1,161	123	84	39	48
1,100 1,200	34	1,152	130	84	45	54
1,100 1 200	27	1,148	124	75	46	66
1,200 1,300	4	1,243	136	86	49	57
1,200 1,300	22	1,250	135	83	50	66
1,200 1 300	28	1,242	127	80	45	58
1 300 1,400	5	1,331	120	79	40	52
1,300 1,400	9	1,331	135	86	47	55
1,300 1,400	13	1,347	121	76	44	63
1 400 1,500	5	1,456	133	89	44	50
1,400 1,500	12	1 437	137	85	50	60
1,400 1,500	8	1,465	128	76	50	66
1,500 1,600	2	1,566	121	85	36	42
1,500 1 600	8	1,542	133	80	58	73
1,500 1,600	4	1,537	129	84	45	54
1,600 1 700	1	1,633	132	70	62	88
1,600 1,700	1	1 658	115	68	47	69
1,700 1,800	1	1 724	140	70	70	100
Total	344					

Comparing the weak and strong halves of the series, which is representative of the subjects who are more likely and those who are least likely to have circulatory inefficiency and cardiac overload, we see that the pulse-pressure percentage of diastolic pressure can offer us no reliable index

In a former work[4] it was shown that the size of the pulse-pressure is influenced by the systolic pressure, and that the systolic pressure is much more responsive even to slight influences, than is the diastolic

pressure We must also take into this consideration the fact that the unit of diastolic pressure has a greater value than the unit in systolic pressure

As time goes on and our observations accumulate and are being interpreted, we believe that more and more importance will be attached to the height of the minimum pressure, and that the height of the maximum pressure will be considered of lesser clinical import than heretofore Certainly the maximum pressure alone is of no value excepting as a starting-point for a guess as to the activity of the circulatory system

SUMMARY

1 In 90 per cent of a series of 656 healthy young men the maximum blood-pressure was under 150 mm Hg It would be still lower if we could eliminate psychic effect

2 In 96 per cent of a series of 338 cases the minimum pressure when read at the fifth phase did not exceed 100 mm Hg

3 In 87 per cent of a series of 312 cases the minimum pressure, when read at the fourth phase did not exceed 100 mm Hg

4 On reexamination of the 13 per cent in the series of 312 cases, we found that 99 4 per cent had a minimum blood-pressure not over 100 mm Hg

5 In 92 per cent of the total 650 cases the minimum pressure did not exceed 100 mm Hg at the time of the first examination

6 In 88 per cent of a series of 629 cases the pulse-pressure ranged between 20 and 70 mm Hg

7 There is no constant relation between the pulse-pressure and the diastolic pressure In 75 per cent of a series of 742 cases the pulse-pressure comprises anywhere from 20 to 80 per cent of the diastolic pressure

8 There is no relation between physical efficiency, which also means circulatory efficiency, and pulse-pressure percentage of diastolic pressure In a series of 344 cases, the poorer half gave practically the same pulse-pressure to diastolic pressure percentage as the stronger half, with their better circulatory system

4502 Fifth Avenue

THE MECHANISM OF CARDIAC DISPLACEMENTS IN PULMONARY TUBERCULOSIS *

MAURICE FISHBERG, M D
NEW YORK

Monographs on tuberculosis which devote considerable space to the physical diagnosis of this disease usually omit a discussion of cardiac displacements which are so frequently encountered in chests affected by infiltration, fibrous degeneration, collapse, shrinkage and cavitation of pulmonary tissue Some mention is occasionally made of dextrocardia as liable to occur in extensive right-sided lesions, but minor degrees of displacement, which are so frequently taking place, are altogether forgotten Some clinicians, however, have observed that dislocation of the heart occurs in pulmonary tuberculosis more often than is generally appreciated Turban[1] said that a displacement of the cardiac dulness several centimeters to the right, with or without displacement of the heart, is such a frequent symptom of moderate contraction of the right apex that he considers it a typical and cardinal symptom of right apical disease of some standing, and that it may be seen in many cases before the appearance of tubercle bacilli in the sputum

In their work on fibroid diseases of the lung, Sir Andrew Clark, Hadley and Chaplin[2] show that of forty-five cases of pulmonary fibrosis, thirty-four had cardiac displacements from a half to 3 or 4 inches out of its normal place Similar conditions were found by these authors in cases of fibroid phthisis

In this country Pottenger[3] has given the subject of cardiac displacement in tuberculosis considerable attention He found dislocation of the heart in some form and to some degree in all cases where either a lessening or increase in the volume of the lung occurs In another paper[4] he gives the following statistics as to its frequency Of 162 hearts examined, 58 were in practically the normal position, 27 were displaced to the right and 77 were displaced to the left Of the first-stage cases

* Read before the Section on Medicine of the New York Academy of Medicine, Oct 21, 1913

* Submitted for publication Nov 6, 1913

1 Turban, K Diagnosis of Tuberculosis of the Lung, London, 1906, p 81

2 Clark, Hadley and Chaplin Fibroid Diseases of the Lung, London, 1894, pp 157, 171

3 Pottenger, F M Displacements of the Heart and Diaphragm in Pulmonary Tuberculosis, *Interstate Med Jour*, *1911*, xviii 605

4 Pottenger, F M The Effects of Tuberculosis on the Heart, THE ARCHIVES INT MED, 1909, iv, 306

one was displaced and 10 were in normal position, of the second-stage cases, 4 were displaced and 17 were in normal position, and of the third-stage cases, 99 were displaced and 31 were in the normal position

In a clinical study of 2,344 cases of tuberculosis made at the Phipps Institute,[5] it was found that the heart was displaced in but 57 instances 12 times downward, 31 times to the left and 14 times to the right, of the last group, 5 were cases of complete dextrocardia On the other hand, Lawrason Brown[6] finds that while in the incipient stage the heart

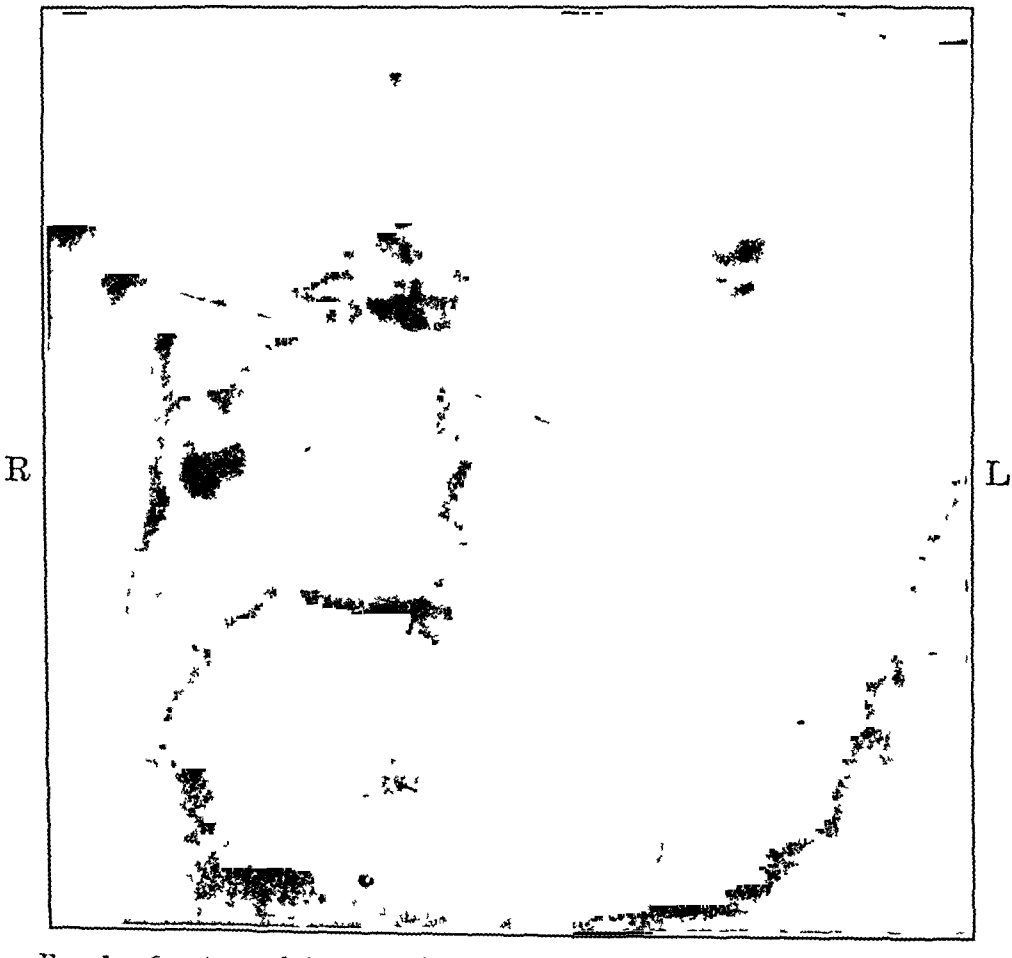


Fig 1—Cavity in left upper lobe, thickened pleura at left base, heart displaced to left and downward Aorta and trachea also dislocated

is rarely appreciably displaced, in many patients in moderately advanced stages the ordinary methods of exploration show some changes in the position of the heart From roentgenographic studies, C L Leonard[7] found in a series of 100 cases of tuberculosis 26 displacements of the heart and aorta, in 17 it was displaced to the right, in 4 to the left,

5 Norris, G W Ann Rep Phipps Institute, 1906, II, 235

6 Brown, Lawrason The Heart in Pulmonary Tuberculosis, Am Jour Med Sc, 1908, cxxxvi, 819

7 Leonard, C L Compensation by Displacement of the Thoracic Viscera in Pulmonary Tuberculosis, Med Rec, 1912, lxxxi, 1

and in 5 a rotation took place with an upward displacement of the heart and aorta, so that the heart assumed an anteroposterior position Leonard is of the opinion that marked alterations in the position of the intrathoracic viscera occur in the earlier stages of tuberculosis without visible changing of the excursion of the thoracic wall Similarly, Francine† found that in many cases of phthisis the heart as well as the aorta, are displaced He found it in 37 per cent of cases, and is of the opinion that dislocation to the right is more frequent than to the left When the pulmonary lesions are of fairly symmetrical character on both sides the heart is more commonly displaced to the right than to the left, according to Francine

In a recent paper Felix Oerl[8] reports 413 cases of tuberculosis in which the position of the heart was carefully studied He found that in 43 per cent of right-sided lesions and 82 per cent of the left-sided lesions the heart was displaced, or more or less shifted to the affected or more affected side It appears from Oerl's investigations that dislocations are more likely to occur toward the left side, a view already expressed by Lawrason Brown [6] This is not because left-sided lesions are more frequent—in fact, they are not—but for anatomical reasons to be discussed later on Ruedinger,[9] on the other hand, states that dislocations of the heart in phthisis is more frequently directed toward the right side than to the left Cases of tuberculosis of the right lung in which no cardiac dulness can be discerned to the left of the sternum are not rare He explains it by the fact that the right lung is the larger lung, and its force of retraction is more powerful, so that traction on the mediastinum will be more effective when pulling it out of its normal location than retraction of the smaller left lung On the other hand, Lawrason Brown points out that it is readily seen that the displacement of the heart may be much more pronounced when the right lung is affected, for the displacement is most often associated with contraction of one lung In right apical lesions of some standing, even when the physical signs are slight, the right border of the absolute cardiac dulness may be found ¾ inch or more to the right Brown states, however that marked displacement of the heart occurs much more frequently to the left than to the right, owing, possibly, to the fact that the tension, when the left lung contracts, is exerted more directly on the heart For these reasons a very frequent displacement of the heart is upward and slightly out-

† Francine, A P The Effects of Tuberculosis on Intrathoracic Relations, Am Jour Med Sc, 1910, cxxxix, 732, Intrathoracic Displacements in Pulmonary Tuberculosis, Bull Johns Hopkins Hosp, 1910, xxi, 244

8 Oerl, Felix Herzverschiebung bei Lungentuberkulose, Beitr z Klin d Tuberk, Brauer's 1913, xxvi, 123

9 Ruedinger E Die Organverlagerung bei Phthise, Beitr z Klin d Tuberk, Brauer's, 1910, xvii, 151

ward The heart, however, may be so greatly displaced to the left, following contraction of the left lung, or pneumothorax, or pleuritic effusion on the right, that the apex may be beyond the left anterior axillary line or in the third intercostal space

My own experience with tuberculous patients is in agreement with that of Pottenger, Brown and Orr In a large number of cases of phthisis in which I looked for the position of the heart while examining the thorax, I found the heart dislocated in such a large proportion that

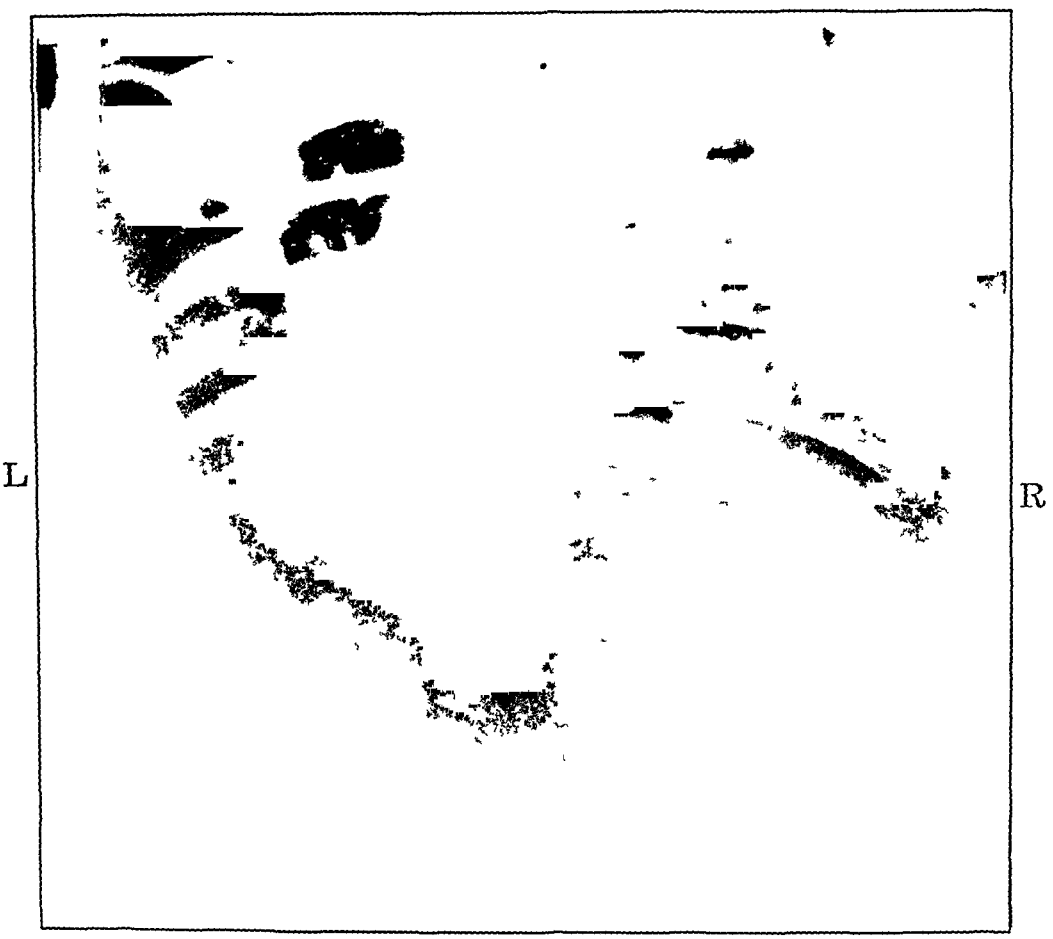


Fig 2—Cardiac displacement to the left and upward, bilateral pulmonary lesion with cavity in left upper lobe Note dislocation of the aorta to the left

I wonder why text-books do not mention it, and why most clinicians overlook it In advanced cases of tuberculosis it is exceptional to find the heart in its normal place It appears, however, that even pathologists have overlooked this fact, or at least have not taken note of it, perhaps because post-mortem conditions do not show vividly the true location of the heart and its relations to the surrounding lungs, especially in minor degrees of displacement Leonard[7] mentions a pathologist who did not believe that displacements could occur so frequently in the earlier stages of the disease, but since his attention was drawn to it, he found dislo-

cations very frequently at necropsies It is only since roentgenography has become a routine method of examination in these cases that the displacements of the heart in tuberculosis are being appreciated It seems to me that by determining the location of the heart, we can, as a rule, determine in which side the tuberculous lesion is located, or in bilateral lesions which side is the older or more serious lesion, that is, accompanied by destruction of lung-tissue and shrinkage

Of 115 consecutive cases of pulmonary tuberculosis in which within the last three months I have taken notes on the location of the heart, I found that in 58, or 50 per cent, the heart was dislocated Of 85 instances with early lesions 31, or 36 per cent, showed cardiac displacements Of these 22 were displacements to the left and 9 to the right In other words, 70 per cent of the displacements were toward the left in early and second-stage cases of tuberculosis Of 29 far-advanced cases, 27 showed cardiac dislocation, of which 15 were to the left and 12 to the right Here, again, we have 55 per cent of displacements to the left

In the tuberculosis ward of Montefiore Home I found that among 18 far-advanced cases with right-sided lesions, or in which the lesion was more extensive in the right side, 14 showed dislocations to the right and 4 to the left, and in one the heart was in its normal location Of 19 left-sided lesions, or lesions more extensive on that side, there were 15 displacements to the left, one to the right, in one instance there was no displacement at all Here the displacements were about equal between the two sides, and, with some exceptions, the shifting of the heart was toward the more affected side In the cases which did not follow this rule, we always found a reason for the anomaly to be explained later on

The mechanism of these dislocations of the heart is an interesting topic for study Experience with intrathoracic tumors, aneurysms, pleural effusions and pneumothorax teaches that the heart is usually displaced toward the healthy side in these cases While during the course of pulmonary tuberculosis we often encounter cardiac displacements of this kind, as in pleurisy, pneumothorax, etc, yet I do not intend to speak about them in this paper What concerns us here are displacements in which the heart is displaced toward the affected side, *par attraction,* some French writers call it In the course of pulmonary tuberculosis, especially in cases running a chronic course, the elastic lung-tissue in the affected region is replaced by fibrous tissue The lung thus loses its elasticity to a great extent, and its motility suffers greatly Shrinkage is another feature, thus making room for any intrathoracic organ that may adjoin the affected part In cases in which there is

also vicarious emphysema of the opposite healthy lung—and they are not at all rare—the mediastinum is pushed toward the affected side Thus in many cases of pulmonary tuberculosis we have in the chest a *vis a tergo* and a *vis a fronte* both effective in displacing the heart toward the affected side

Within the pericardium the heart is free and smoothly movable, otherwise it could not pulsate regularly and rhythmically The pericardium is connected above with the deep cervical fascia by a prolongation of

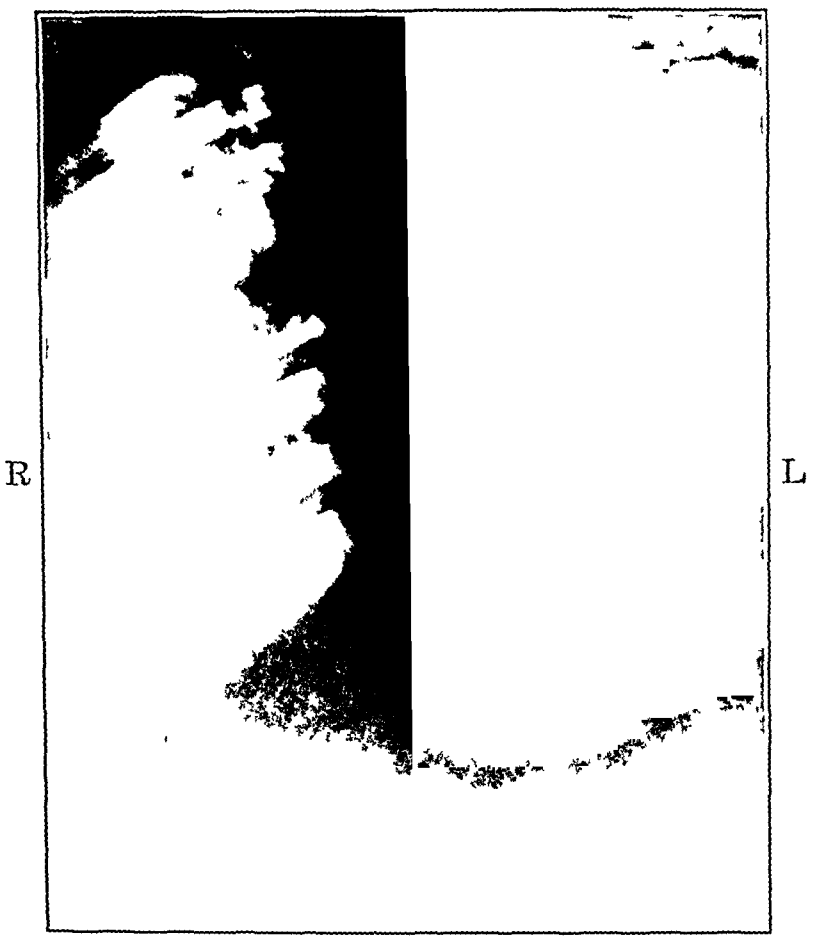


Fig 3—Tuberculous pleurisy and cavitation of left lung, heart displaced to the left and downward, apex-beat in left axillary line

the fibrous portion through the sheaths over the great vessels Two bands of fibrous tissue—known as the sternopericardial ligaments—connect the front of the pericardium above and below with the posterior surface of the sternum Below, the pericardium is connected with the central tendon of the diaphragm closely and intimately It is also firmly bound to the caval openings, but loosely attached to and easily separable elsewhere If its oblique position from right to left, as well as its strong attachment to the diaphragm are borne in mind, it is clear that it requires great force to move the heart to the right, while to the left

it is more easily shifted When either traction from the left side or pushing from the right side occurs, and especially when both forces are operative, the only resistance to be overcome is the upper attachment to the great vessels which is yielding, as is evident from the frequent dislocation of the aorta, trachea and even the larynx in cases of phthisis But when the forces involved are effective in displacing the heart to the right, its intimate attachment to the diaphragm has also to be overcome It is for this reason that dislocations to the right are more often seen in far-advanced cases of phthisis, especially such as are complicated by, or which follow, adhesive pleurisy, while dislocations to the left may take place in the early stages of the disease

That the base, in spite of its attachment to the great vessels, is more movable than the apex of the heart, has been proved experimentally and also by roentgenographic studies of cases of pleural effusion by Bard,[10] Pitres,[11] Sallé[12] and many others It has been found that when fluid is injected into the left pleura there is no displacement of the heart till at least 1,000 c c are injected, and only then the heart moves a little to the right, but no more than 2 or 3 cm When the injection of fluid is continued, the apex hardly moves held as it is firmly by the central tendon of the diaphragm, but the base does shift to the right, and the whole body of the heart is moved forward, nearer to the anterior chest wall In cases of left-sided pleurisy with effusion, Carrière[13] has found confirmatory evidence through roentgenographic studies An effusion of from 700 to 1,000 c c does not at all displace the heart, the apex remains in its normal place, and only when the patient assumes an erect posture, or reclines on his right side, does the apex move from 2 to 4 cm to the right, nearing the median line In effusions of from 1 to 3 liters the long axis of the heart becomes vertical, the apex is lowered and shifted to the median line, and with an effusion of 3 liters it is moved as far as the xyphoid appendix When the effusion exceeds this quantity, the heart is shifted completely to the right, but it is not swung over, retaining as it does the normal direction of its long axis from right to left and from above downward—the apex remains at the xyphoid appendix and the base is shifted to the right hemithorax Torsion is never observed

10 Bard Refoulement du coeur à droite et dextrocardie congénitale, Lyon méd, 1892, lxxi, 583, 1893, lxxii, 1, Refoulement du coeur à droite dans les épanchements pleuraux, Méd moderne, 1897, viii 185

11 Pitres Les signes physiques des épanchements pleuraux et en particulier sur les deplacements du coeur, Arch clin de Bordeaux, 1896, v, 1, 65, 241

12 Sallé De déplacements du coeur par les épanchements pleuraux gauches au point de vue experimental et clinique, Thèse de Paris, 1908 1909

13 Carrière Déplacement du coeur et des organs abdominaux dans les épanchements pleuritiques, Presse méd, 1898, vi, 349

Of course, it is often different with cases of phthisis with strong fibrous bands and pleuropericardial adhesions which immobilize either the apex or the base and keep it in that position In case the base is immobilized, the apex may move through traction by some other fibrous bands, and the heart assumes a vertical position within the chest, or the axis may be reversed, running from left to right but this is exceedingly rare According to the amount, density, location and direction of the adhesions and pulmonary retraction that may be present in a given case of phthisis, the heart may be dislocated upward or downward, to

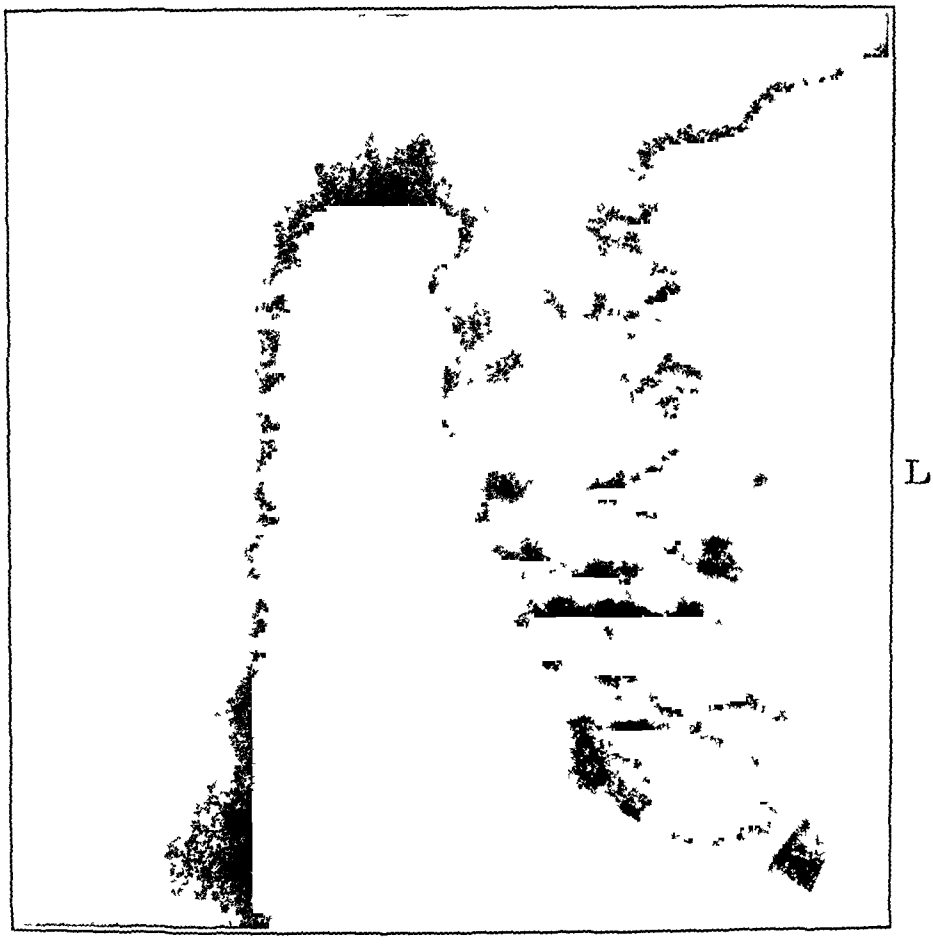


Fig 4—Cardiac dislocation to the right with rotation on its sagittal and vertical axes, bilateral pulmonary lesion, adhesive pleurisy in right hemithorax

the right or to the left The apex may be lifted up to an extent as to bring the heart in a horizontal position resting on the elevated diaphragm Clinical experience shows many of these anomalous dislocations when we look for them

The usual location of tuberculous lesions is at the apices, and retraction and scar formation occurs more often in the upper part of the lungs and pleura, thus pulling the heart upward by fibrous bands running to its base from the affected lung or pleura When the traction comes

from the left upper lobe the heart easily yields and moves upward and to the left, when traction is exerted from the right apex, the effect depends on the extent and kind of lesion in the lung In cases with strong fibrous bands running from the right pleura to the pericardium, especially when there is also cavitation in the right lung making place for its reception—here also Nature abhors a vacuum—and the left lung is emphysematous, encroaching with its bulk on the right side of the thorax, pushing the mediastinum toward the right, all obstacles are overcome more or less and the heart moves to the right side, in extreme cases we may find under these circumstances complete dextrocardia with the apex-beat to the right of the sternum Shrinkage of the lung takes place in both a horizontal and a vertical direction, and thus not only the mediastinum, but also the diaphragm is often dislocated Strictly speaking, elevation of the diaphragm in one side will push the heart upward and to the opposite healthy side, but inasmuch as fibrosis and retraction of the lung not only pulls up the diaphragm vertically, but retracts the lung horizontally, the heart is usually attracted toward the affected side When there is a pulmonary cavity and emphysematous lung on the opposite healthy side, it is evident that the dislocation will be toward the affected side

In *tuberculosis* the position of the heart is consequently greatly influenced by the position of the diaphragm An elevation of the right half of that muscle tends to shift the heart to the left, elevation of the left half of the diaphragm first raises the apex of the heart, bringing it into a horizontal position, and when the height of the left half of the diaphragm greatly exceeds that of the right half, there may occur a dislocation of the heart to the right When pleuropericardial adhesions are strong and dense they may pull the heart to the left and upward, as we see in many cases of phthisis, especially those following adhesive pleurisy of the left side

Older writers spoke of a form of cardiac dislocation in which that organ, fixed at its base by the great vessels, was swung over to the right side like a door on its hinges This has been found erroneous, as has been shown in the excellent anatomical, clinical and experimental studies of Bard,[10] Pitres,[11] Sallé[12] and others, but we do know that rotation on its sagittal and vertical axes often does take place In the normal position of the heart the left ventricle is nearer the chest wall than the right, which is turned to the back As Oeri[8] points out, traction exerted on the right side brings the right ventricle nearer the chest wall, while the apex is moved away from it, traction on the left side may increase the obliquity of its position and pull the heart completely toward the left Cavitation in the left upper lobe, combined with fibrous bands running between the pleura and pericardium, dislocates the heart not only

to the left but also upward, and this is greatly assisted by the upward displacement of the diaphragm, as was already mentioned It must also be borne in mind that the left half of the diaphragm is easily movable [14] Traction toward the right, if not very strong, may turn the heart on its sagittal, and at times on its vertical axis, and as a result we find an increase in the dulness to the right of the sternum, because the base of the heart has approached the chest wall without much shifting, while the apex, moved as it is backward, cannot be found on inspection and percus-

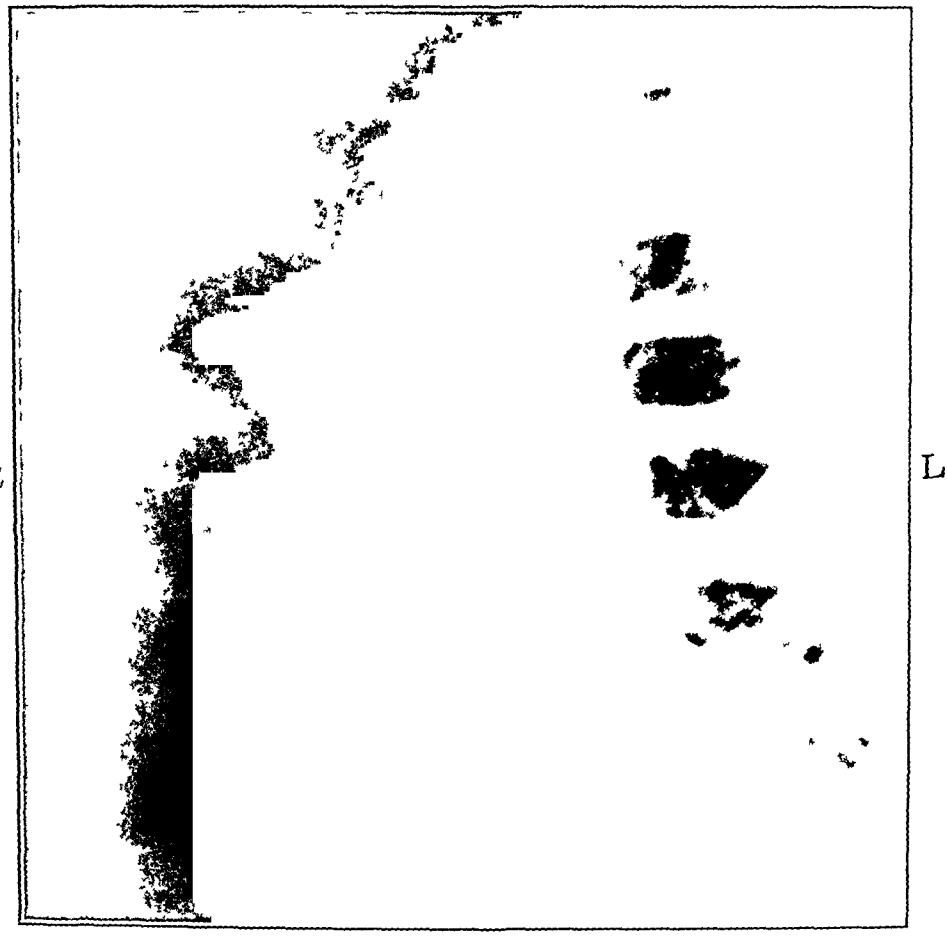


Fig 5—Shifting of heart to the right with rotation on its sagittal axis in an extensive right sided tuberculous lesion

sion This explains some of the cases of vertical hearts which we find so often in roentgenograms when examining advanced cases of tuberculosis

When we have two forces combined, as in traction on the right side due to dry pleurisy with massive adhesions, pulmonary fibrosis, long-standing tuberculous pleurisy with formation of false membrane, pleuro-pericardial adhesions which immobilize the heart and fix it in an abnormal position, and an emphysematous lung in the left hemithorax

14 Sahli Diagnostic Methods, Second American edition, p 251

making place for itself by encroaching across the sternum to the right, we may find complete and permanent dextrocardia In these cases there is no rotation of the heart with swinging all the way around on its point of fixation at the great vessels, as was formerly thought This would be incompatible with life As has been shown by recent experimental investigations at the necropsy-table and with the Roentgen ray, the heart is in these cases pushed over to the right hemithorax *in toto,* the base is usually displaced to the same extent as the apex, and it retains its natural oblique position in the chest—its long axis is directed from above downward and from right to left But instead of lying to the left of the sternum, it is located to the right of the median line of the body Carefully studied cases of dextrocardia of this type have been reported by Baird,[10] Gallard,[15] Lortat-Jacob and Laignel-Lavastine,[16] Barbier[17] and many others I encounter these cases comparatively often and at the Montefiore Home I have had four under observation during the last three years They are very rare in acute cases, running speedily to termination by death, but in those which run a chronic course extending over many years, dextrocardia may occur in extensive right-sided lesions. They also occur in cases of pleurisy which are strongly adhesive from the beginning, and follow rapid absorption of a pleural exudate when the lung does not reexpand quickly to fill up the space left vacant by the disappearance of the fluid In these cases the pleura forms a thick shell around the collapsed lung into which it sends forth thick fibrous bands When the latter contract they may drag the mediastinum, thus filling up vacant space in the right hemithorax

I want to say a few words about those rare cases of dextrocardia in which the base of the heart remains in nearly its normal position while the apex is found in the right mammillary line, or even further toward the axilla, the direction of its long axis is from above downward and from left to right It was this sort of case of dextrocardia that formerly led to the belief that in some dextrocardias the heart is swung over to the right side As has already been stated, such dextrocardias do not exist because the kinking of the great vessels would promptly extinguish life These forms of dextrocardia usually occur in the following manner An effusion into the left pleura pushes the entire mediastinum to the right and keeps it there until the fluid is absorbed or removed In the vast majority of cases the heart then returns to its normal location

15 Gallard, M L Dextrocardie par attraction, Bull et mém Soc méd d. hôp de Paris, 1908, xxv 634

16 Lortat Jacob and Laignel-Lavastine Dextrocardie acquise par retraction pleuro pulmonaire avec caverne tuberculeuse, Bull et mem Soc méd d hôp de Paris, 1906, xxiii, 814

17 Barbier Un cas de dextrocardie au cours d'une sclerose pulmonaire tuberculose droite, Bull et mém Soc d hôp de Paris, 1900, xxii, 187

on the left side but in some rare cases in which the right pleura is affected, the apex of the heart, while sojourning in the right hemithorax, is bound down by strong pleuropericardial adhesions, so that while the base, which is free, moves back to its normal place, the apex remains fixed in its abnormal position Such a case has been reported in detail by Chalier and Rebattu,[18] and in one case that I have seen this was the only plausible explanation for the anomalous position of the heart

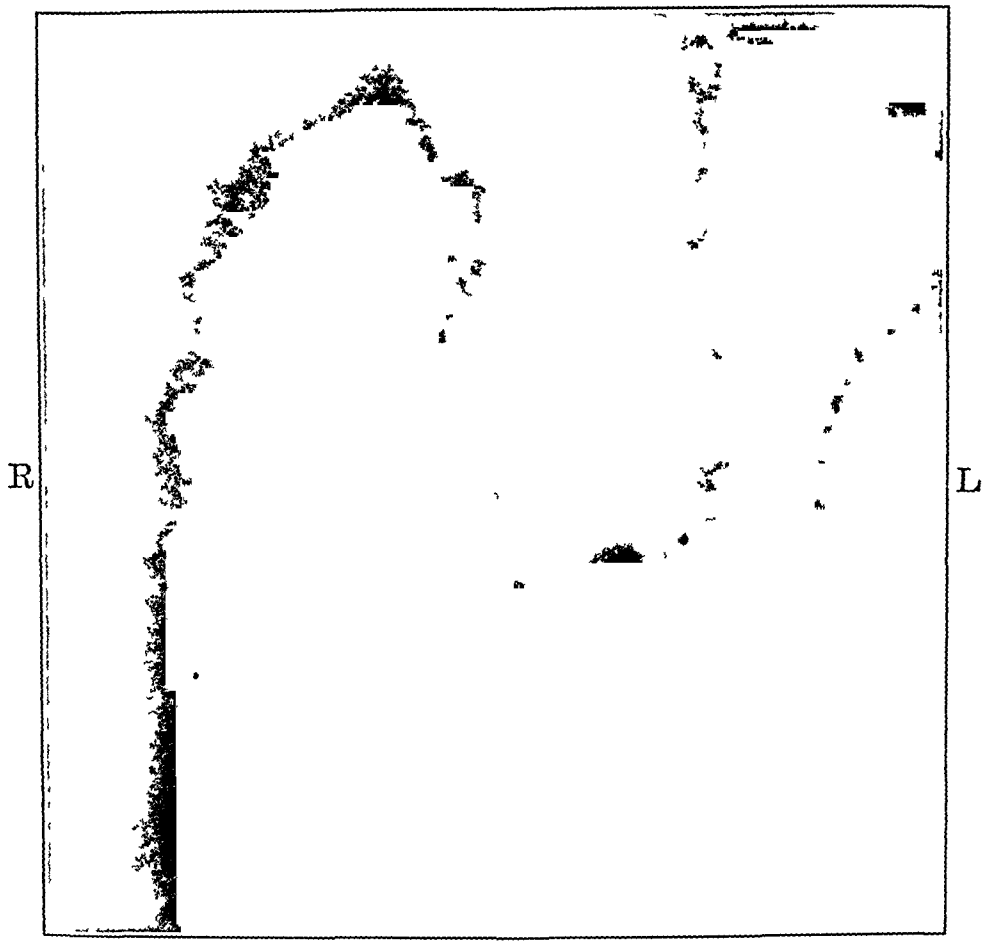


Fig 6—Acquired dextrocardia

While in most cases of cardiac dislocation in tuberculosis the heart is displaced toward the affected side and in bilateral lesions to the side in which the older and more serious lesion is found, there are many exceptions Pleuropericardial adhesions often prevent dislocation of the heart, which ordinarily would be pulled or pushed out of place At times we encounter extensive infiltration, or a large cavity in the right upper or middle lobe, while the heart is dislocated 1 or 2 inches to the left In these anomalous cases I almost invariably found that in addi-

18 Chalier, J, and Rebattu, J Des dextrocardies acquises, Paris méd, 1912, p 365

tion to the lesion in the right lung, there was also a thickened pleura at the left base, adherent to the left side of the pericardium which pulled and fixed the heart toward the left side

The routine methods of physical exploration show the location of the displaced heart in phthisis easily and vividly, but in many cases the diagnosis is very difficult, and occasionally almost impossible The side of the heart adjoining the healthy lung is easily made out by percussion, but the cardiac dulness at the side adjoining the affected lung merges with the dulness of the infiltrated and consolidated lung-tissue or thickened pleura, and is difficult to separate by any method of percussion The fluoroscope and the roentgenographic plate also fail at times to show a definite outline of the borders between the heart and lung Indeed, I have found in many cases that the orthodiascope was of no value, because extensive infiltration and thickened pleura have obscured all outlines which in normal subjects show such a clear picture of the heart and lungs The diagnosis of dextrocardia is also sometimes difficult Of course, from complete transposition of the viscera it is easily distinguished when the location of the liver, spleen, etc, is noted, but when we find the heart on the right side, the apex beating to the right of the sternum and the liver, spleen, etc, in their normal locations, the question may arise whether we are not dealing with one of those rare cases of congenital transposition of the heart in which this is the only organ displaced This is especially true of cases in which the heart was dislocated by a latent pleurisy during infancy of which the patient can give no account The electrocardiograph may give us positive information in these cases, as by a study of the records from the different leads the axis of the heart may readily be determined We had a case at the Montefiore Home in which the question arose whether it was congenital or acquired, and the Roentgen ray could give no positive diagnostic clues The electrocardiogram taken by Dr B S Oppenheimer, however, showed a normal tracing

While percussing and auscultating these chests, it is to be borne in mind that the heart is not only shifted from left to right, but in many cases there is also some rotation of that organ on its vertical or sagittal axis—the base approaching the chest wall, while the apex is shifted away from it As a result it often happens that the point at which the impulse of the apex is apparently found is altogether the impulse of the right ventricle Occasionally we find two points with impulse beneath—one in the second or third right intercostal space, and another near the xyphoid cartilage As a general rule, we may say that when on inspection palpation and auscultation we find the heart-beat at only one point, it is the base or the right ventricle which is visible and palpable, because in these cases the apex is turned away from the anterior

chest wall, when two points are found with impulses, the one to the left represents the apex and the one to the right represents the base for reasons just made clear

Even in moderate dislocations we find that the cardiac sounds undergo important modifications While it is undoubtedly true that the great frequency of the accentuation of the second pulmonic sound, as well as the impulse at the second left intercostal space near the sternum, are due to pulmonary retraction, yet in a large number of cases the dislocation of the heart and rotation on its vertical axis are responsible for it

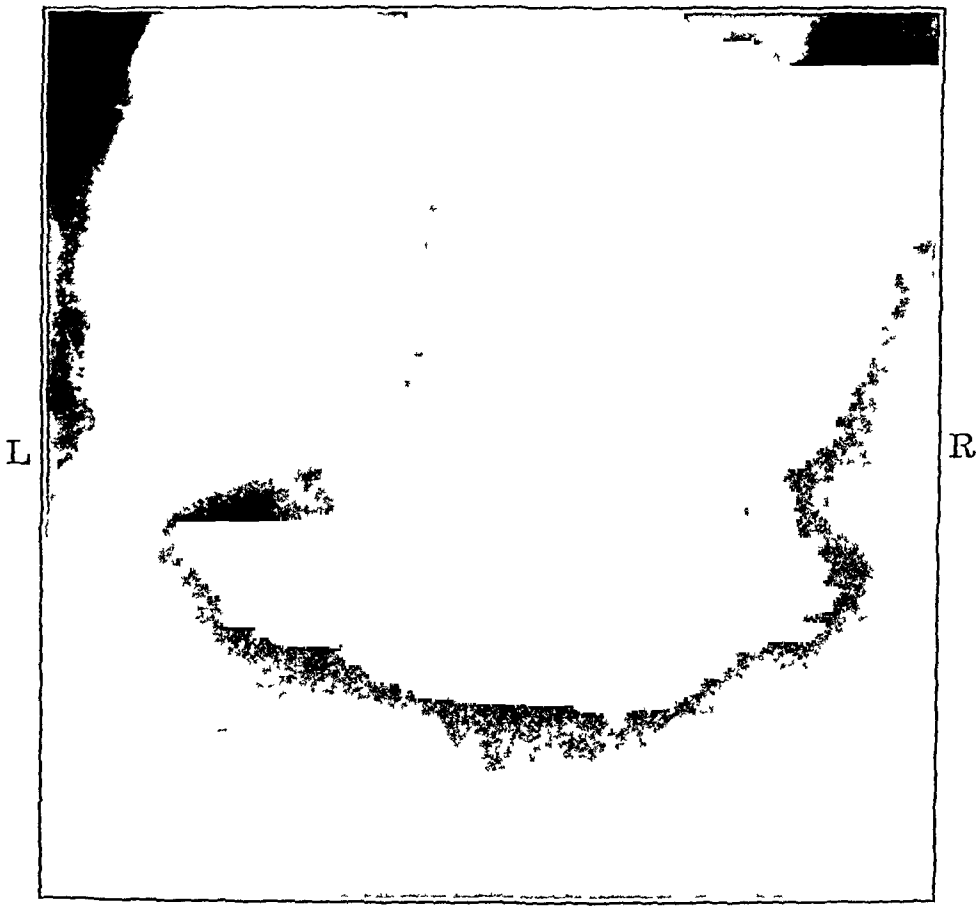


Fig 7 —Acquired dextrocardia, large cavity in right upper and middle lobes, thickened right pleura, vicarious emphysema of left lung

in a great measure Similarly, often no apex-beat can be seen or felt, although percussion and auscultation show that it is located in its normal place In some this is due to an emphysematous lung covering the apex, but in others rotation with shifting backward is responsible At times we find that although the apex is shifted to the left, the cardiac impulse remains within the mammillary line, which is also due to rotation of the heart on its vertical or sagittal axis The fact is that two cases of cardiac dislocation showing the same signs and symptoms are very rare, which is clear when we bear in mind the various directions

in which traction by fibrous bands may be exerted and the different locations of shrinking pulmonary tissue, cavitation, compensatory emphysema, as well as the condition of the pleura in its relation to the pericardium, and above all the position of the diaphragm It has been my impression that in displacements to the left, the second pulmonic sound is very frequently accentuated, while dislocations to the right are more often accompanied by an accentuation of the aortic sounds In both cases it may be due to either greater conductivity of densely infiltrated lung tissue, to obstruction of the pulmonary circulation as in emphysema, or to the fact that the base of the heart has been moved nearer to the anterior chest wall

The effects of cardiac dislocations on the circulation and on the general condition of the patient depend on several factors, especially the rapidity with which it is accomplished, the distance it is dislocated out of its normal position, and also the mode of displacement Most dislocations are found in very old and chronic cases, mainly in fibroid phthisis, and the effects on the circulation come on slowly and insidiously, the heart adapting itself more or less to its new position Pottenger[3] observed a case in which the heart moved more than 1 inch to the right within a few days' time owing to the formation of a large cavity in the right lung near the heart border The symptoms were extreme weakness, dyspnea, rapid heart-action and a tendency to syncope, loss of appetite and nausea, especially when the patient changed his position In a case under my care, the heart was dislocated upward and to the left about 1½ inches out of place within two weeks, and the patient, a boy aged 15, suffered from extreme prostration, dyspnea and tachycardia, and also showed signs of cerebral anemia

In all the chronic cases of tuberculosis in which the heart is appreciably dislocated, tachycardia is a constant symptom, but tachycardia is a cardinal symptom of phthisis without cardiac displacement, and has been attributed by various authors to pressure on the vagus by swollen bronchial glands, or fibrous scars, etc, and also to the toxemia of the disease In cardiac dislocation, however, we have an added mechanical factor In my experience annoying tachycardia is extremely rare in afebrile cases of phthisis, unless there is also cardiac dislocation, when it becomes an important symptom, and the least exertion results in severe palpitation, dyspnea, weakness, etc Ruedinger believes that there are undoubtedly forms of tuberculosis in which the rapid heart-action is primarily due to disturbances in the mechanism of the heart as a result of the dislocation When we examine these cases fluoroscopically, we find obvious reasons for cardiac embarrassment in the displacement of the thoracic organs Some healed cases of tuberculosis

remain permanently with tachycardia, and inasmuch as toxemia cannot be held responsible for the rapid heart-action in inactive cases, the dislocation of the heart may be held accountable

SUMMARY AND CONCLUSIONS

Cardiac dislocations are very frequently found in pulmonary tuberculosis In far-advanced cases of phthisis it is exceptional to find the heart in its normal location, over 75 per cent of cases showing displacements of the heart In moderately advanced cases between one-third and one-half show displacements, and even some early cases are thus affected

The dislocation is usually produced by traction exerted from the affected side Pulmonary collapse and retraction, cavitation and fibrous bands running from the pleura to the pericardium drag the heart out of its normal place Pressure exerted by a vicariously emphysematous lung on the opposite side greatly assists in displacing the heart to the affected side, or, in bilateral lesions, to the side which has the older or more serious lesion In many cases we can determine in which side the tuberculous lesion is located or in bilateral lesions in which side the older or more extensive lesion is located, by determining the location of the heart

Dislocations take place more often toward the left hemithorax than toward the right for various anatomical reasons mainly, however, because of the oblique position of the heart from right to left, and the strong attachment of the mediastinum to the central tendon of the diaphragm, which greatly counteracts traction exerted from the right pleura and lung As a result we find that about two-thirds of all displacements are toward the left side In far-advanced cases the displacements are about equal between the two sides of the chest, and dextrocardia is not uncommon Acquired dextrocardia is almost invariably of tuberculous origin

In many cases of phthisis the heart is not only dislocated, but also rotated on its sagittal or vertical axis In the normal position the cardiac apex is nearer the chest wall than the base Traction exerted on the right side of the mediastinum may bring the base of the heart nearer the chest wall and push or pull the apex away from it Elevation of the diaphragm also has a great influence on the position of the heart, and together with pleuropericardial adhesions may dislocate the heart in any manner—upward, downward, to the right or left, may bring it in a horizontal or vertical position, etc

In the majority of cases the diagnosis of displacement can be made by percussion, auscultation and roentgenoscopy, but in some cases only the side of the heart adjoining the healthy lung can be mapped out by percussion, while on the opposite side its dulness merges with that of the pulmonary or pleural lesion and cannot be differentiated easily, and at

times even the Roentgen ray is of no avail In dextrocardia a good guide is the cardiac impulse When we find only one point with an impulse, it is usually the right ventricle that pulsates and not the apex because in these cases the latter is turned away from the anterior chest wall, if we find two points with pulsation the one to the left represents the apex and the one to the right the base or the right ventricle

While tachycardia and dyspnea of phthisis are usually due to toxemia of the disease the cardiac displacements are also responsible to some extent Many patients cured of tuberculosis remain with permanent cardiac embarrassment because of the displacement of the heart

1337 Madison Avenue

The Archives of Internal Medicine

Vol XIII MAY, 1914 No 5

ANIMAL CALORIMETRY. PAPER VIII THE ALLEGED INFLUENCE OF THE ADRENALS ON DIABETIC METABOLISM*

GRAHAM LUSK
WITH THE ASSISTANCE OF
J A RICHE
NEW YORK

The discovery by Blum[1] that administration of epinephrin caused glycosuria at once suggested that its action might be due to an effect on the pancreas This hypothesis was tested by Herter and Wakeman[2] who found that direct application of epinephrin solutions to the pancreas caused the appearance of sugar in the urine Benedicenti[3] and Pemberton and Sweet[4] showed that epinephrin injections diminish the flow of pancreatic juice The last-named authors suggested the possibility that the "internal secretion" of the pancreas was likewise inhibited and therefore diabetes resulted Edmunds,[5] however, set forth that the inhibition of the pancreatic flow was due to a reduced blood-supply to the gland brought about by vasoconstriction

An elaborate theory as to the action of epinephrin was promulgated by Falta, Eppinger and Rudinger,[6] a theory which has had the support of von Noorden,[7] whose pupils these authors were According to this theory, epinephrin inhibits the internal secretion of the pancreas, thereby preventing the oxidation of sugar; it stimulates the thyroid to increased

* This article and the six following, viz., those of Graham Lusk and J A Riche, E R Baldwin, J A Hartwell, J P Houget and Fenwick Beckman, C F Craig, Horst Oertel, C W Field, and W W Herrick are contributions in commemoration of the hundredth anniversary of the foundation of the medical department of Yale University, and will be republished in book form

* Submitted for publication March 3, 1914

* From the Physiological Laboratory, Cornell University Medical College, New York

1 Blum Deutsch Arch f klin Med, 1901, lxxi, 146

2 Herter and Wakeman Virchow's Arch f path Anat, 1902, clxix, 179

3 Benedicenti Arch ital d biol, 1905, xlv, 1

4 Pemberton, Ralph, and Sweet, J E The Inhibition of Pancreatic Activity by Extracts of Suprarenal and Pituitary Bodies, THE ARCHIVES INT MED, 1908, i, 628

5 Edmunds Jour Pharmacol and Exper Therap, 1911, ii, 559

6 Falta, Eppinger and Rüdinger Ztschr f klin Med, 1908, lxvi, 1.

7 Von Noorden New Aspects of Diabetes, 1912

times even the Roentgen ray is of no avail In dextrocardia a good guide is the cardiac impulse When we find only one point with an impulse, it is usually the right ventricle that pulsates and not the apex because in these cases the latter is turned away from the anterior chest wall, if we find two points with pulsation the one to the left represents the apex and the one to the right the base or the right ventricle

While tachycardia and dyspnea of phthisis are usually due to toxemia of the disease the cardiac displacements are also responsible to some extent Many patients cured of tuberculosis remain with permanent cardiac embarrassment because of the displacement of the heart

1337 Madison Avenue

The Archives of Internal Medicine

Vol XIII MAY, 1914 No 5

ANIMAL CALORIMETRY PAPER VIII THE ALLEGED INFLUENCE OF THE ADRENALS ON DIABETIC METABOLISM*

GRAHAM LUSK
WITH THE ASSISTANCE OF
J A. RICHE
NEW YORK

The discovery by Blum[1] that administration of epinephrin caused glycosuria at once suggested that its action might be due to an effect on the pancreas This hypothesis was tested by Herter and Wakeman,[2] who found that direct application of epinephrin solutions to the pancreas caused the appearance of sugar in the urine Benedicenti[3] and Pemberton and Sweet[4] showed that epinephrin injections diminish the flow of pancreatic juice The last-named authors suggested the possibility that the "internal secretion" of the pancreas was likewise inhibited and therefore diabetes resulted Edmunds,[5] however, set forth that the inhibition of the pancreatic flow was due to a reduced blood-supply to the gland brought about by vasoconstriction

An elaborate theory as to the action of epinephrin was promulgated by Falta, Eppinger and Rudinger,[6] a theory which has had the support of von Noorden,[7] whose pupils these authors were According to this theory, epinephrin inhibits the internal secretion of the pancreas, thereby preventing the oxidation of sugar, it stimulates the thyroid to increased

* This article and the six following, viz., those of Graham Lusk and J. A Riche, E R Baldwin, J A Hartwell J P Houget and Fenwick Beekman C F Craig, Horst Oertel, C W Field, and W W Herrick are contributions in commemoration of the hundredth anniversary of the foundation of the medical department of Yale University, and will be republished in book form

* Submitted for publication March 3, 1914

* From the Physiological Laboratory, Cornell University Medical College, New York

1 Blum Deutsch Arch f klin Med, 1901, lxxi, 146

2 Herter and Wakeman Virchow's Arch f path Anat, 1902, clxix, 479

3 Benedicenti Arch ital d biol, 1905, xcv, 1

4 Pemberton, Ralph, and Sweet, J E The Inhibition of Pancreatic Activity by Extracts of Suprarenal and Pituitary Bodies, THE ARCHIVES INT MED, 1908, i, 628

5 Edmunds Jour Pharmacol and Exper Therap, 1911, ii, 559.

6 Falta, Eppinger and Rüdinger Ztschr f klin Med, 1908, lxvi, 1

7 Von Noorden New Aspects of Diabetes, 1912

activity, thereby causing an increase in protein metabolism, and it causes a transformation of sugar into fat

Although not one of these statements is true, the theory has possessed a charm from which many have not been able to free themselves, and it has also been productive of work which has shown the matter in its proper light

Thus, Ringer[8] rendered dogs diabetic with phlorhizin according to a method originally perfected at the Yale Medical School by Reilly, Nolan and Lusk,[9] and after freeing the dogs of glycogen by shivering, found that administration of epinephrin caused no increase of either sugar or of nitrogen in the urine Epinephrin, therefore, does not cause the production of sugar from fat nor does it increase protein destruction through thyroid stimulation

The present paper deals with the remaining question whether or not epinephrin glycosuria is due to an inhibition of the internal secretion of the pancreas, which thereby renders the organism truly diabetic in the sense that there is a decreased power to oxidize glucose

This problem has been considered by other authors, but their published results are conflicting Thus Hári[10] administered epinephrin intraperitoneally and intravenously to curarized dogs from twenty-four to thirty-six hours after the last food ingestion, and found that the respiratory quotient was somewhat increased above the level existing prior to the injection of the drug The increase in the respiratory quotient was usually coincident with a rise in blood-pressure Wilenko[11] employed rabbits under urethane anesthesia and found that after the ingestion of sugar the respiratory quotients of normal animals ranged between 0 90 and 0 98 Wilenko found, however, that if epinephrin were administered, the respiratory quotient did not rise under the influence of sugar ingestion From this he drew the conclusion that epinephrin prevents the oxidation of carbohydrate in the organism

Fuchs and Roth[12] administered epinephrin subcutaneously to three persons, one of whom had Addison's disease, and found an increase in the respiratory quotient Similar results which were reported by Falta[13] have been described in full by Bernstein[14]

The question of the inhibition of carbohydrate metabolism through epinephrin seemed to be in an unsettled state and one which might be

8 Ringer Jour Exper Med, 1910, xii, 105
9 Reilly, Nolan and Lusk Am Jour Physiol, 1898, i, 395
10 Hári Biochem Ztschr, 1911, xxxviii, 23
11 Wilenko Biochem Ztschr, 1912, xlii, 49
12 Fuchs and Roth Ztschr f exper Path u Therap, 1912, x, 187
13 Falta Die Erkrankungen der Blutdrusen, 1913, p 428
14 Bernstein Ztschr f exper Path u Therap, 1914, xv, 86

determined by experiments with the respiration calorimeter constructed by Dr H B Williams in this laboratory [15]

It may be added that Allen,[16] using other methods, has reached the conclusion that epinephrin glycosuria is without relation to diabetes

EXPERIMENTAL

The work was accomplished with two dogs Both animals were given 0 001 mg of epinephrin (adrenalin hydrochlorid of Parke, Davis and Co) per kilogram of body weight administered subcutaneously The

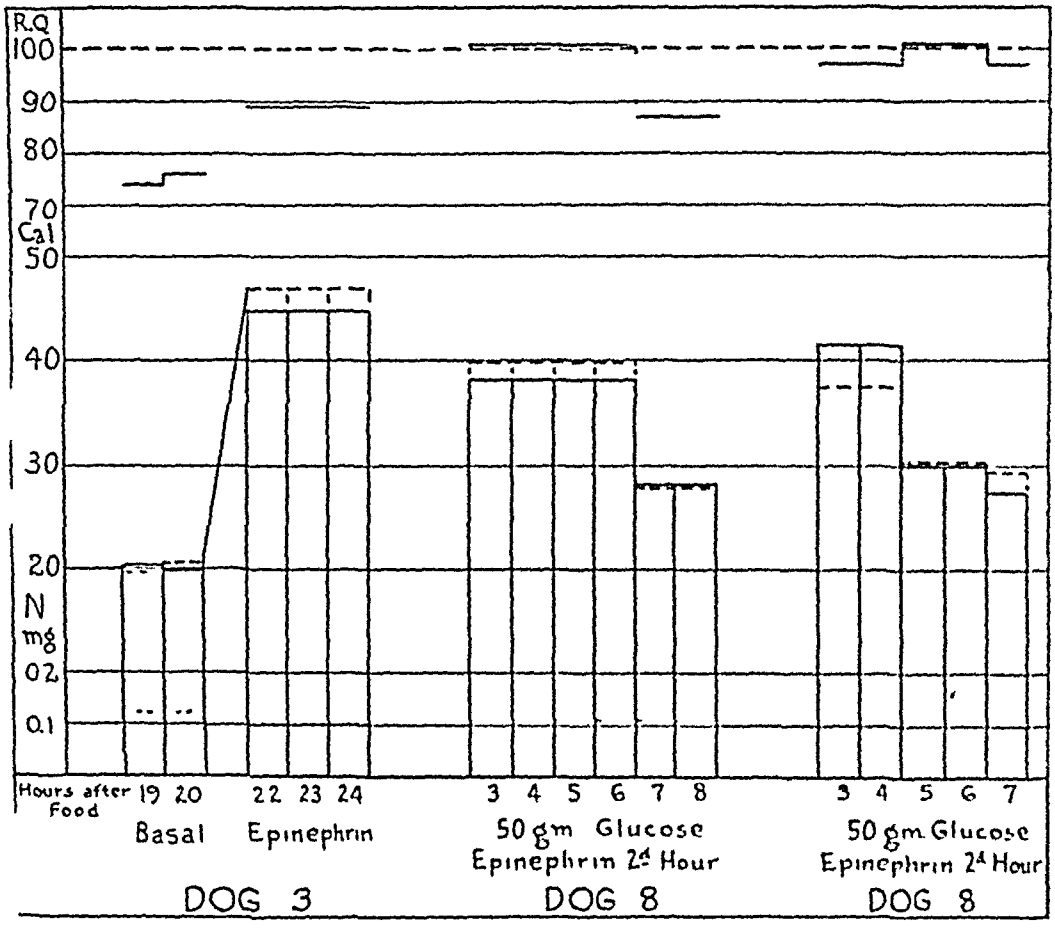


Chart showing the influence of epinephrin on metabolism Solid lines represent calories calculated, broken lines calories found, and dotted lines milligrams of nitrogen

animals were brought into the calorimeter as soon after the injection as possible The urine of the period was collected by catheterization The dogs were very active while under the influence of the drug, exhibiting unrest and polypnea as has been described by various authors Dog 3, which had rested quietly in the calorimeter on twenty-one previous occasions, became quite violent after receiving epinephrin, incidentally

15 Williams Jour Biol Chem , 1912, xii, 317
16 Allen Glycosuria and Diabetes, 1913, p 1063

removing the thermometer from the rectum and chewing it into pieces The rectal thermometer was therefore not used in the experiments with Dog 8 On this account the direct and indirect measurements of heat are not so close together as they might be A wire netting was made to surround the dog bed so that the valuable internal adjustments of the calorimeter should not suffer damage

TABLE 1

	Time	CO_2 gm	O_2 gm	R Q	H_2O gm	Urine N gm	Non Protein CO_2 gm	Non Protein O_2 gm	Non Protein R Q
Dog 3 Exp 21	a m p m 11 00 12 00	6 39	6 13	0 76	8 12	0 120	5 27	5 12	0 7
	p m p m 12 00- 1 00	6 38	5 94	0 78	7 01	0 120	5 26	4 93	0 7
Exp 22	p m p m 2 00 5 00	48 13	39 18	0 89	65 95	0 315	45 18	36 52	0 9
Dog 8 Exp 1	a m p m 11 00 3 00	61 16	43 39	1 02	103 25	0 428	57 16	39 77	1 0
	p m p m 3 00 5 00	19 67	16 50	0 87	35 74	0 214	17 67	14 69	0 8
Exp 2	a m p m 11 00 1 00	31 75	23 92	0 97	51 97	0 354	28 44	20 93	0 9
	p m p m 1 00 3 00	24 30	17 18	1 03	45 07	0 354	20 99	14 19	1 0
	p m p m 3 00 4 00	10 58	7 90	0 97	20 05	0 177	8 93	6 40	1 0

Necrosis of the skin always follows subcutaneous injections of epinephrin given in these quantities, but no permanent injury ensues Dog 3 retains a scar on the back at this writing, nearly a year after the treatment, and has experienced fifty experimental periods in the calorimeter

The full details of all the experiments are given in Table 1, and are graphically illustrated in the accompanying chart

A THE EFFECT OF EPINEPHRIN INJECTIONS ON THE BASAL METABOLISM

The *basal metabolism* in this series of papers has been taken as the average heat production during two or three hours of absolute rest following the eighteenth hour after the ingestion of a maintenance ration Benedict has lately described this metabolism as being "post-

absorptive" Since the basal metabolism or *Grundumsatz* may remain at the same level during months or even years, it seems unnecessary to change its name It appears to be a desirable expression representing a uniform base line which is immediately affected by changes induced by food ingestion, mechanical work, by variations in external temperature, by drugs or by disease

TAILS OF EXPERIMENTS

Calories				Body Temperature			Weight, Mg	Behavior of Dog	Remarks
ein	Non Protein	Total Calc	Total Found	Start	End	Dif			
3 18	16 99	20 17	19 53	37 68	37 39	—0 29	12 15	Quiet	Basal metabolism
3 18	16 48	19 66	20 60		37 58	+0 19		Quiet	
		39 83	40 13						
8 35	125 90	134 25	140 62	38 00	38 83	+0 83	12 15	Very active	12 mg epinephrin, 1 13 p m
11 52	141 45	152 97	159 32				11 0	Very active	50 gm glucose, 9 a m 11 mg epinephrin, 10 15 a m
5 76	50 37	56 13	55 56					Active and quiet	
		209 10	214 88						
9 38	73 76	83 14	74 88				11 0	Very active	50 gm glucose, 9 a m 11 mg epinephrin, 10 16 a m
9 38	50 71	60 09	60 24					Active and quiet	
4 69	22 61	27 30	29 51					Quiet and movement	
		170 53	164 63						

The basal metabolism of Dog 3 (weight = 12 1 kg) was determined between 11 a m and 1 p m , beginning eighteen hours after the ingestion of a standard diet consisting of 100 gm of biscuit meal, 100 gm beef heart, 20 gm lard and 10 gm of bone ash. This diet contains 688 calories, of which 137 calories (20 per cent) are in protein, 251 (36 per cent) in fat and 300 in carbohydrate (44 per cent) The quantity of carbohydrate in the biscuit meal amounted to 73 gm [17]

At 1 p m the dog was removed from the calorimeter, catheterized, and at 1 13 p m received a subcutaneous injection of 12 c c 1 1,000 epinephrin solution The animal was returned to the calorimeter and the experimental work was resumed at 2 p m The animal was extremely active throughout the experimental period of three hours Between 2

17 Lusk Jour Biol Chem , 1912, xiii, 186.

p m and 3 52 p m (when the rectal thermometer ceased recording) the body temperature of the dog increased 0 83°

Table 2 shows the influence of epinephrin injection on the basal metabolism of the dog

This experiment shows that the injection of epinephrin does not increase the nitrogen in the urine During the period of the basal metabolism, calculations[18] made in the light of the respiratory quotients show that 0 8 gm of glucose was oxidized per hour, amounting to 16 per cent of the total heat evolved, while, during the period of epinephrin influence, 7 4 gm of glucose were oxidized per hour during three hours, yielding 62 per cent of the total heat produced The oxidation of sugar was increased ninefold The results are to be explained by the well-authenticated fact that the administration of epinephrin causes hyperglycemia[19] through discharge of sugar from the glycogen stores of the body[20] The 22 gm of glucose oxidized during the three-hour period corresponded to less than 0 2 per cent of glycogen in the well-nourished dog, a very moderate quantity The urine of the experimental period was free from sugar but after the usual standard diet had been administered to the dog following its removal from the calorimeter, the urine became charged with sugar

The increase of 125 per cent in the metabolism may be entirely attributed to muscular movements

This experiment shows that *epinephrin administered twenty-one hours after food ingestion does not prevent the oxidation of glucose in a well-nourished dog*

B THE EFFECT OF EPINEPHRIN INJECTIONS ON THE METABOLISM AFTER GLUCOSE INGESTION

Two experiments were performed on Dog 8, a small, fat animal, weighing 11 kg The standard diet was given daily at 5 o'clock beginning on the evening of April 29 As the dog was not trained to lie quietly in the calorimeter the basal metabolism was not determined It could scarcely have exceeded 20 calories per hour

At 9 a m 50 gm of glucose in 150 c c of water were given to the dog, and at 10 15 a m 11 c.c epinephrin, 1 1,000, were given subcutaneously, the experiment began at 11 a m The results may be condensed as in Table 3

Experiment 1 shows that the urinary nitrogen was 0 107 gm per hour which is not above the usual output of nitrogen during the period

18 For the method of calculation see Williams, Riche and Lusk Jour Biol Chem, 1912, xii, 356

19 Vosburg and Richards Am Jour Physiol, 1903, ix, 35

20 Drummond and Noel Paton Jour Physiol, 1904, xxxi, 92

TABLE 2—Experiments 21 and 22 on Dog 3, Showing the Influence of Epinephrin on the Basal Metabolism

	Time	N in Urine gm	R Q	Calories				
				Protein	Fat	Carbo-hydrate	Total Calc	Total Found
Basal metabolism	11 a m to 12 m	0 120	0 76	3 18	14 56	2 42	20 17	19 53
Basal metabolism	12 m to 1 p m	0 120	0 78	3 18	12 36	4 12	19 66	20 60
							39 83	40 13
Average per hour		*0 120*		*3 18*	*13 46*	*3 27*	*19 92*	*20 07*
Epinephrin metabolism	2 to 5 p m	0 315	0 89	8 35	42 81	83 09	134 25	140 62
Average per hour		*0 105*		*2 78*	*14 27*	*27 70*	*44 75*	*46 87*

TABLE 3—Experiments 1 and 2 on Dog 8, Showing the Influence of Epinephrin During a Period of Glucose Absorption

Time	N in Urine gm	R Q	Calories				
			Protein	Fat	Carbo hydrate	Total Calculated	Total Found
11 a m to 3 p m	0 428	1 02	11 52		141 45	152 97	159 32
3 to 5 p m	0 214	0 87	5 76	20 55	29 82	56 13	55 56
						209 10	214 88
11 a m to 1 p m	0 354	0 97	9 38	2 76	71 00	83 14	74 88
1 to 3 p m	0 354	1 03	9 38		50 71	60 09	60 24
3 to 4 p m	0 177	0 97	4 69		22 61	27 30	29 51
						170 53	164 63

of basal metabolism of a dog nourished on the standard diet employed in this laboratory In Experiment 2 the nitrogen elimination was found to be 0 177 gm per hour, an abnormally high figure, undoubtedly due to the necrosis of the skin brought about by the previous injection of epinephrin

In Experiment 1 there was an elimination of 7 9 gm of glucose in the urine during seven hours following the injection of epinephrin Deducting this amount from 50 gm actually ingested, there remain 42 1 gm available for oxidation The respiratory quotient of the six hours of the experiment was 0 98 Calculating as before, it appears that 46 gm of glucose, or 7 7 gm per hour, were actually oxidized to furnish 171 27 calories liberated from carbohydrate in the organism This represents 82 per cent of the total heat production during the period Since the metabolism experiment did not commence until two hours after the administration of the glucose, it is evident that the carbohydrate oxidized during the six hours of the experimental period counted some glycogen drawn from the body's store of that material

It may be argued that epinephrin injection might produce an effect on the pancreas which might render it more susceptible if the injection were given a second time The experiment was, therefore, repeated in all its essentials on the same dog five days later The dog had received the standard diet at 5 p m daily during the interim

In Experiment 2 there was an elimination during six hours of 11 1 gm of urinary glucose, leaving available for metabolism 38 9 gm of 50 gm ingested During the experimental period of five hours, the respiratory quotient averaged 0 99 The heat produced from carbohydrate was 144 32 calories, which is the equivalent of 38 gm of glucose, representing an hourly destruction of 7 6 gm In this instance, 85 per cent of the total calories of metabolism were derived from glucose

Comparing these results with former work[21] accomplished on a normal dog which had received 50 gm of glucose, it becomes evident that *the injection of epinephrin in the dog has no influence on the metabolism of carbohydrate during the period of glucose absorption*

The cause of epinephrin glycosuria is best explained by the work of Ritzmann[22] who found that sugar appeared in the urine only when the dose of the drug was sufficient to cause vasoconstriction Such a condition may bring about anemia of the tissues, under which circumstance there is always a production of lactic acid, a discharge of glucose from the glycogen repositories a marked increase in the quantity of blood-sugar and consequent glycosuria Lactic acid may stimulate the respiratory center causing polypnea The oxygen absorption is such, however,

21 Lusk Jour Biol Chem, 1912, xiii, 27
22 Ritzmann Arch f exper Path u Pharmakol, 1909, lxi, 231

that anemia of the tissues can only be relative, and is not such as to prevent a large oxidative energy production

SUMMARY

The subcutaneous injection of epinephrin in a well-nourished dog twenty hours after the ingestion of a standard mixed diet sufficient for maintenance caused an increase of the respiratory quotient from 0 77 to 0 89 From the former quotient it may be calculated that 16 per cent of the heat production was at the expense of glucose, while from the latter it appears that 62 per cent had that source Before the injection of epinephrin, 0 8 gm glucose was oxidized per hour, and after the injection 7 4 gm per hour The dog became muscularly active about half an hour after the administration of the drug

The subcutaneous injection of epinephrin in a dog which had received 50 gm of glucose resulted, in one experiment, in a respiratory quotient for a six-hour period of 0 98, and a combustion of glucose amounting to 7 7 gm per hour, corresponding to 82 per cent of the heat production of the period On repetition of the experiment six days later, the respiratory quotient was found to be 0 99 during a period of five hours and the oxidation of glucose amounted to 7 6 gm hourly, being the equivalent of 85 per cent of the total calories produced

The heat produced from the oxidation of 7 5 gm of glucose is 28 calories, and the basal metabolisms of the dogs did not exceed 20 calories per hour This is suggestive that epinephrin does not cause a diminution in the power of the organism to oxidize glucose

The theory that epinephrin causes a production of sugar from fat, decreases the power of the organism to oxidize glucose through inhibition of pancreatic function, and stimulates the thyroid so that protein metabolism is increased, is untenable in any of its particulars

Cornell University Medical College, New York City

EXPERIMENTAL STUDIES ON THE BLOOD-SERUM OF COWS IMMUNIZED AGAINST TUBERCULOSIS

'SENSITIZATION" OF LIVING TUBERCLE BACILLI'

EDWARD R BALDWIN, M D
SARANAC LAKE, N Y

The experiments given in detail in this contribution relate to some calves that were immunized in 1902 and 1903 by intravenous inoculations of human tubercle bacilli The results of various serum reactions, including complement deviation, agglutination and precipitation, were published in 1904[1] Bactericidal and bacteriolytic tests, which were briefly described at that time,[2] were also performed with culture bacilli

Nearly all the calves were killed at the conclusion of the first-mentioned experiments Calf 5 was, however, retained for more than ten years, being repeatedly inoculated with cultures and extracts of tubercle bacilli These were always of the human type, so that the actual test of immunity-inoculation of bovine bacilli was never made Nevertheless, from the well-known protection acquired by calves under similar treatment, it is fair to assume that this animal was at least relatively immune

The further study of immune serums in general, and of this animal especially, appeared of sufficient importance to indicate its retention, as in the course of time additional knowledge of serology was developed

So far as can be discovered from a search of the literature, no instance is mentioned of an immunized bovine being observed over so long a period At least, no observations of the blood-serum have extended over such a long time, to the best of our knowledge It seemed, therefore a sufficient reason for publishing some of the experiments, although they are chiefly of interest to laboratory workers in tuberculosis

Some of the experiments have already been reviewed[3] Of these, many were tests for opsonin or bacteriotropin, but the difficulty of deter-

* From Saranac Laboratory for the Study of Tuberculosis, Dr E L Trudeau, Director

* Submitted for publication March 3, 1914

1 Studies from the Saranac Laboratory, Jour Med Research, 1904, xii, 215

2 Studies from the Saranac Laboratory, Jour Med Research, 1904, xii, 226

3 Opsonins in Tuberculosis, Tr Assn Am Phys, 1907, p 517

mining the varying content of these substances by a normal standard serum was not overcome, owing to the agglutination It was shown that a specific bacteriotropin was present, but it could not be measured

Previous experiments had failed to produce antitoxic serums for tuberculosis, so that no extensive trial was made of the neutralizing effect of the immune cow-serums Neither was the trial made of serum injections on animals or man with the hope of restraining infection as in the more recent experiments of Neporoschny,[4] Ruppel and Rickman,[5] Calmette and Guérin,[6] Pawlowsky,[7] and Sata[8] The harmful effects of foreign serums in producing anaphylaxis have long been recognized as a drawback to serotherapy Notwithstanding this, Neporoschny, Ruppel and Sata asserted that their serums, when injected into guinea-pigs, retarded infection Calmette and others found similar serums harmful and the disease aggravated

Our interest was centered in the plan of "sensitizing" living tubercle bacilli with immune serums It was hoped that by saturating tubercle bacilli or their products with the immune antibody from serums, a better vaccine or tuberculin might be produced The serum *per se* could thus be washed out but its specific virtues retained, anchored to the bacilli

Within the past three years such a vaccine, made from B E. tuberculin sensitized by Ruppel's serum, has been patented in Germany by F Meyer[9] and has been employed as a mild-reacting tuberculin under the designation "S B E" It is said to have diminished toxicity in comparison with plain B E Neporoschny also used such a vaccine for immunizing dogs, whose serum in turn was employed in the treatment of tuberculosis[10] We could not show any lessening of toxic effects of B E by our experiments

As our work was with living, intact bacilli and with cow-serums of lower antibody content than the horse- and mule-serums used by Ruppel, it is not possible to say that our results are contradictory in this respect, though it would so appear in the methods here described

In the details which follow, some protocols of our first attempts at bacteriolysis are included for the sake of completeness

4 Neporoschny, S D (quoted by Pawlowsky) Ztschr f Tuberk, 1911, xvii, 8

5 Ruppel, W G, and Rickman, W Ztschr f Immunitätsforsch, 1910, vi, 344

6 Calmette, A, and Guérin, C Ann de l'Inst Pasteur, 1911, xxv, 629

7 Pawlowsky, A D Ztschr f Tuberk, 1911, xvii, 1

8 Sata, A Ztschr f Tuberk, 1911, xviii, 1, 1913, xx, 1

9 Meyer, F Berl klin Wchnschr, 1910, No 20, p 926

10 Neporoschny, reviewed by Piel P Beitr z klin d Tuberk, xxi, 311

BOVINE IMMUNIZATION, WEAK-VIRULENT HUMAN BACILLI

Bactericidal Influence of Immunized Serum from Heifers Nos 4, 5 and 6 on Cultures

The following notes were made in 1903 of the first tests made with the serum of No 5 together with others (Nos 4 and 6) The animal was then 11 months of age and weighed 240 pounds Three months previously (Dec 1, 1902), the first immunizing inoculation of weak-virulent living human tubercle bacilli, culture R1, had been administered. The dose was 0 0015 gm intravenously The serum was obtained twenty-four hours after bleeding

EXPERIMENT 74.—March 19, 1903 Culture on agar of human tubercle bacilli, K1, moist growth, March 8, 1903, emulsified in normal saline, centrifuged one minute, gave a strongly opaque emulsion Took 0 5 c c tubercle bacilli emulsion, and 1 5 c c fresh active serums of No 2 (normal) and Nos 4, 5 and 6 (immunized), incubated one hour, centrifuged, decanted serums and planted four tubes each from the sediments

CULTURE GROWTHS IN EXPERIMENT 74

Date	Serum No 6	Serum No 4	Serum No 5	Serum No 2	Control
March 21	3 good 1 contam	1 fair 2 slight 1 neg	Good on all	2 fair 2 slight	2 good
April 20	4 good	2 good 2 slight	Good on all	Good on all	2 good

Result.—No evidence of bactericidal influence on cultures of tubercle bacillus after treatment by calf serums

This experiment was repeated three weeks after the second inoculation of Calves 4 5 and 6 with 0 025 gm R1 tubercle bacillus

EXPERIMENT 96.—April 2, 1903 Culture human tubercle bacilli K1 agar, March 19 1903 Rubbed in mortar with normal sodium chlorid, centrifuged two minutes weak emulsion Serums fresh, active, from bleeding April 1 Took 1 c c emulsion 2 c c each, Serums 2, 4, 5 and 6 Incubated two hours, centrifuged A, sediment transferred to agar plates, B, part of the sediments were treated a second time for nine hours with 2 c c of fresh serums, then plated on agar

April 8 A, good growth on all in microscopic colonies, no contaminations, B, slight growth in Serums 5 and 6

Result.—No bactericidal influence clearly evident by this method

Other attempts to demonstrate bactericidal effects on cultures were made with the culture R1 and also with virulent cultures at this time but without success Contaminations were frequent in some tests, in most the cause of failure was the uncertainty of growth after soaking in the serums The method was therefore limited to the use of Culture K1, which resembled the avian bacillus, though said to have been the direct descendant of Koch's first culture from human sources

Animal inoculations of tubercle bacilli treated with Serums 2 and 6 immunized had already failed to reveal any bactericidal effect, consequently no further attempts were made with Serum 5 until repeated injections of tubercle-bacilli products over a long period had been made

Immunized Cow 5, Injected with Water-Extracts of Tubercle Bacilli

During 1904 and 1905 large quantities of filtered water-extracts of tubercle bacilli were injected into Cow 5, both subcutaneously and intravenously The amounts varied from 25 c c to 175 c c, and frequent tests of the serum were made for specific agglutinin, precipitin and complement deviation

The agglutination titer[11] reached only 1 to 100 and 0 025 c c deviated complement but the precipitin titer was low—1 to 4

Experiment 237—Oct 12, 1905, Bactericidal test by inoculation Cow 5 weight 850 pounds Tuberculin test 0 5 c c No reaction

November 14 Bled 100 c c aseptically, defibrinated with iron shavings, centrifuged 20 c c for serum

B 10 c c defibrinated blood + 1 c c emulsion tubercle bacilli virulent culture human H37, Nov 7 1905 Four oese to 4 c c normal saline

S 5 c c serum only + 0 5 c c as before

C 1 5 c c normal saline + 1 c c as before

All incubated seventeen hours, no contaminations, centrifuged, B and S diluted, B to 6 c c, S to 4 c c

November 15 Inoculated two guinea-pigs intraperitoneally with each culture as follows

Culture	Amount Each in c c	No	Weight gm	Duration of Life, Days	Average Days
C	1 25	1	150	39	41½
		2	150	44	
S	2	3	475	13	14½
		4	445	16	
B	2	5	425	13	22½
		6	460	22	

Result—All the animals died of peritoneal tuberculosis with some generalization The animals inoculated with blood and tubercle bacilli seemed to have a more rapid infection than the controls

From this and other experiments we concluded that prolonged exposure of tubercle bacilli to normal (0 8 per cent) saline solution, even in the absence of light, has a weakening action on the vitality of the tubercle bacillus

BOVINE IMMUNIZATION WITH VIRULENT HUMAN INOCULATION

The immunization methods hitherto employed appeared to produce little effect on the serum reactions of our bovines It was at least not evident in agglutinin, precipitin and complement-deviation tests to an

11 With Koch's agglutination emulsion, diluted 1 to 1,000

extent that was expected More virulent human bacilli were then tried with the hope of stimulating greater reaction responses to tuberculin injections and presumably bacteriolytic antibodies

To this end, Cow 5, now being full-grown and in excellent condition, weight 850 pounds was given Nov 14, 1905, 0 020 gm (moist weight) in 10 c c normal saline, virulent human culture H37 broth from Oct 19, 1905 intravenously in the jugular The highest temperature recorded in the next twenty-four hours was 102 6 F No ill effects were noticeable but the cow was left untreated for nearly a year except for occasional bleeding

Cow No 15—A fresh cow, 2 years old, weight 650 pounds, tuberculin test negative, had also been inoculated Oct 30, 1905, with (0 02 in 10 c c normal saline) virulent human culture H38 from broth Oct 15, 1905, intravenously in the same way as Cow 5 No reaction occurred from the inoculation until the following day, when the temperature reached 103 F at 5 p m Subsequently for three weeks the temperature varied from 103 to 104 F at the same hour

The animal gradually resumed her normal condition, but had evidently foci from the inoculation, though not a progressive infection After a year she was given a tuberculin test simultaneously with Cow 5, and reacted to 0 5 c c O T to 41 2 C (106 F), indicating the persistence of some unabsorbed tubercles resulting from the inoculation Two days after the test both Cow 15 and Cow 5 were bled for a preliminary test of the serums as to the bactericidal power

EXPERIMENT 237 B—Oct 24, 1906 Bled Cows 5 and 15 two days after tuberculin test, Cow 15 reacting, Cow 5 not reacting

October 26 Serums separated Culture of virulent human tubercle bacilli H38 xx Small portion rubbed in mortar + 15 c c normal saline, centrifuged ten minutes Supernatant emulsion very weak and contains isolated tubercle bacilli

A 0 5 c c emulsion + 2 c c normal saline
B 0 5 c c emulsion + 2 c c active Serum 5
C 0 5 c c emulsion + 2 c c active Serum 15

All incubated aseptically twenty four hours at 37 5 C (99 5 F)

October 27 Centrifuged A, B and C, pipetted B and C and discarded serums, added fresh serums, respectively, Serums 5 and 15 Incubated forty eight hours

October 29 Repeated and incubated twenty four hours

November 1 Removed and pipetted all tubes without shaking them The sediments were examined microscopically for contaminations and for evidences of lysis in the tubercle bacilli All tubes uncontaminated no certain evidence of lysis although some bacilli were vacuolated

November 1 Inoculated all sediments into guinea pigs, subcutaneously right groin as follows

	Pig	Weight gm	November 30 Right Inguinal Nodes
A	1	375	Not noticeable
B	2	335	Enlarged to size of wax bean
C	3	310	Enlarged to size of wax bean

December 7 Pig 1 killed thirty seventh day Few small nodules in lungs No caseation in spleen and glands Smears from spleen, tubercle bacilli negative

December 9 Pig 3 (370 gm) killed thirty-ninth day by tuberculin injection 0 5 c c O T Enlarged right inguinal nodes with caseation, cold abscess in groin, spleen enlarged and congested along its edge, a few tubercles seen outlined in the liver

December 10 Pig 2 killed fortieth day, tuberculin injection, B F , no reaction to 0 5 c c In good condition, weight 370 gm Enlarged and slightly caseous right inguinal nodes, no abscess, mesenteric nodes enlarged and caseous, spleen enlarged and beginning caseation, numerous small caseated areas in liver, none in lungs

Result—Clearly no evidence of bactericidal effect on the part of serums On the contrary, the control emulsion in normal saline apparently failed to infect the animal, whereas repeated treatment with fresh active serums did not destroy enough bacilli, if any, to prevent infection, although but few bacilli were present

Immunizing Treatment, Cows 15 and 5†*

Cow 15 was given frequent injections of tubercle bacilli water-extracts subsequent to the preceding experiments They were injected subcutaneously and intravenously and no fever reactions were noted from 20 c c doses

During the immunization of Cow 15 with water-extracts of tubercle bacilli, there was a different method tried on Cow 5 This animal gave no reaction to 0 5 c c O T . Oct 22, 1906, a year after the inoculation of virulent human tubercle bacilli The serum agglutination titer was 1 to 33, practically that of a normal cow Injections were therefore commenced of a preparation of tubercle bacilli residue left after extraction with water and 10 per cent sodium chlorid followed by alcohol and ether This residue was emulsified in normal saline, 1 0 gm (dry) to 300 c c, and injected in increasing doses from 1 5 c c. to 35 c c subcutaneously and intravenously Only slight reactions were produced from 25 c c of the emulsion subcutaneously After three weeks' treatment the agglutinin titer reached 1 to 75 An experiment was then made with the serum in comparison with that of a healthy normal cow, using a very weak emulsion of tubercle bacilli for inoculation

* The agglutination titer of Serum 15 never exceeded 1 to 100, nor was any precipitin obtained The animal was slaughtered December, 1907, and the lungs and other viscera were normal in appearance The lung hilus lymph-nodes were also normal except that the largest showed a white cicatrix in its center No inoculations were made with it so that its tuberculous source could only be surmised

† In March, 1907, some experiments were undertaken with rabbits inoculated intravenously with virulent human tubercle bacilli treated with Serums 15 and 5, and that of Cow 16 (normal), for a control, together with broth and normal saline controls After the inoculations the ten rabbits were killed at stated intervals during the succeeding six hours with the object of studying the reaction of the tissues to the "sensitized" bacilli The pathologic study was never made, but other observations about that time tended to diminish the interest in the result

EXPERIMENT 237 C.—Dec. 3, 1906. *Guinea-Pig Inoculations.*—Cow 5 bled seven days after an injection of 25 c.c. residue subcutaneously, without fever reaction. Normal Cow 16 bled at the same time.

December 4. Serum agglutination test. Serum 5, 1:75; Serum 16, 1:50.

December 4. Prepared a weak emulsion of virulent human culture H39 from serum slant, Nov. 12, 1906, in normal saline centrifuged. Supernatant emulsion pipetted; tubercle bacilli scarce in smears.

Used 0.5 c.c. emulsion + No. 1. Control, 3 c.c. normal saline.

Used 0.5 c.c. emulsion + No. 2. Control, 3 c.c. Serum of Cow 16 inactivated at 65° C. (149° F.).

Used 0.5 c.c. emulsion + No. 3——— 3 c.c. active Cow-Serum 16.

Used 0.5 c.c. emulsion + No. 4——— 3 c.c. active Cow Serum 5.

All incubated twenty four hours, centrifuged Nos. 3 and 4.

December 5. Changed the serums for fresh active serums by carefully avoiding sediment in pipetting.

December 6 and 7. Same procedure as followed December 5.

December 8. Centrifuged all tubes, pipetted all except Tube 1. Added 3 c.c. normal salt solution to each tube except Tube 1. When well shaken and stirred, smears were made from each for examination. Tubercle bacilli were seldom found, but no morphologic change was seen. No contaminations noted. The contents of the tubes were then inoculated into twenty guinea-pigs, five to each series. Each tube was filled to 3.5 c.c. and 0.7 c.c. inoculated subcutaneously in the right groin of each animal.

Jan. 13, 1907. Thirty sixth day. Inguinal nodes examined and palpated.

Series 1. No enlargement.

Series 2. Two show hyperplasia, one doubtful.

Series 3. Three show hyperplasia, two doubtful.

Series 4. All show hyperplasia.

January 15. Thirty eighth day. One pig in each lot killed by puncture. They revealed progressively more disease from Series 1 to 4. No disease found in Series 1.

January 16. Thirty ninth day. All the other animals were killed and compared. Series 1 revealed no tuberculous lesions to eye. Small gelatinous or translucent nodules found subpleurally in lungs of Series 3, 4 and 5. Inoculations of sections of lung into two guinea-pigs were negative after thirty three days (nodules probably lymph-nodes).

Series 2. Pig 1, deep inguinal nodes caseated, iliac nodes slightly diseased, otherwise negative.

Pig 2, deep inguinal, iliac and lumbar nodes caseated, spleen involvement doubtful, lungs have translucent nodules as in Lot 1.

Pig 4, superficial and deep inguinal and iliac nodes caseated, lumbar nodes and spleen slightly diseased.

Pig 5 (pregnant near term), deep inguinal and iliac nodes caseated, lumbar nodes and spleen doubtful.

Series 3. Pig 1 (pregnant near term), inguinal, iliac, lumbar and lung hilus nodes caseous, nodules beginning in spleen which is slightly enlarged.

Pig 2, same as Pig 1, only more pronounced disease in the spleen.

Pigs 3 and 4 same as 1 and 2, except beginning disease in liver. Series 3 distinctly more diseased than Series 2.

Series 4. Pigs 1, 2, 3 and 5 show marked caseation and hyperplasia of inguinal, iliac and lung-hilus nodes, enlarged and nodular spleens, beginning areas in liver and lobular, translucent areas in lung. All of this series was very uniformly diseased and distinctly advanced beyond the preceding series.

Result.—There was a definitely greater infection in animals of the immunized serum Series 5 than in the controls. A similar difference existed between the animals of the control Serum 16, active and inactive, respectively, while the saline control apparently failed to infect at all, as in previous experiments.

Discussion

It again appeared that our immunized cow-serum was more potent in favoring infection than in restraining it The conditions of the last experiment were so carefully controlled that the accuracy of the observations was unquestionable The interpretation of them was more puzzling Several suggestions were considered

1 Were the bacilli reproducing in the serums during the four days' successive incubation? This is hardly probable, as the growth in inactivated serum would have been more than in active serum presumably exerting its alexin power

2 Did not the greater agglutinating action of the immunized cow-serum act to protect the vitality of the bacilli by coating them with agglutinins, and by reason of their clumping, render them more difficult of access to the leukocytes of the guinea-pig than single bacilli? This appeared more plausible but leaves no ground to believe that the serum plays an important part in the bacteriolysis of tubercle bacilli The inference that cellular elements are more involved in the duty of overcoming the tubercle bacilli was plain Our preliminary experiments with defibrinated blood (Experiment 237A) did not cover this point satisfactorily Hence a repetition of the work seemed indicated with more care in securing tubercle bacilli free from clumps

Guinea-Pig Inoculation with Virulent and Weak Tubercle Bacilli

Experiment 237 C2.—Feb 1, 1907 Serum and Leukocytes Cows 5 and 16 were bled, the former seven days after the last dose of tubercle bacilli residue, 20 c c The serum was separated at once from defibrinated blood and used to repeat the previous experiments, very few tubercle bacilli being employed An emulsion of virulent culture of human tubercle bacilli H39 serum slant from Dec 31, 1906, was prepared and centrifuged twelve minutes It was diluted and the control examined for clumps before being used Very few tubercle bacilli (1 or 2) were found in an entire smear and only rarely in clumps With this very weak emulsion the serums were added as follows

I

A 0 5 c c control emulsion + 3 c c Normal saline
B 0 5 c c control emulsion + 3 c c Serum 5 (active)
C 0 5 c c control emulsion + 3 c c Serum 16 (active)
D 0 5 c c control emulsion + 3 c c Serum 5 (inactive) at 65 C

All incubated one hour

In addition to the preceding, an attempt to increase the virulence of tubercle bacilli was made in the following manner

An emulsion of weak-virulent human culture R1, serum slant from Jan 23, 1907, was prepared in the same way and the following mixtures made

II

A 0 5 c c emulsion + 3 c c Normal saline
B 0 5 c c emulsion + 3 c c Serum 5 (active)

All tubes were incubated four successive days, those containing serums being centrifuged, pipetted and fresh serum added each day All were centrifuged

February 5 and the serums pipetted Before dilution smears were made of equal (measured in an opsonic pipet) quantities of the sediments to note any evidence of changes in the number and form of the bacilli

Careful staining and examination failed to show any marked differences In some slides no tubercle bacilli were found, in others two or three in an entire slide Series 2 had ten to fifteen to a slide, the emulsion of R1 being stronger than H39

To the sediments were added normal saline to make 3 5 c c in each tube Thirty guinea-pigs were inoculated in series of five for each test, 0 7 c c being injected into the right groin

The cows were meanwhile bled again aseptically February 2, directly into the tubercle bacilli emulsion H39 prepared the previous day, as follows

III

A 0 5 c c emulsion + 5 c c normal saline and 1 per cent sodium citrate solution + 5 c c fresh blood from Cow No 5

B 0 5 c c emulsion + 5 c c normal saline and 1 per cent sodium citrate solution + 5 c c fresh blood from Cow 16

C 0 5 c c emulsion + 5 c c 1 per cent sodium citrate solution (control)

After mixing and incubating six hours the blood-tubes were centrifuged, pipetted and the leukocyte layers transferred to tubes to which 1 c c sodium citrate solution was added to prevent coagulation These were also incubated four days Smears made of the final centrifuged cells showed numerous leukocytes, but few phagocyted tubercle bacilli in polynuclear leukocytes A few extracellular tubercle bacilli but no clumps were noted in casual search in a few fields There was no apparent contamination The sediments were then inoculated into six guinea-pigs in series of two for each tube

Necropsies

February 11, sixth day *No 4, Series I D* (Serum 5, inactive) died Nothing abnormal found Agar and serum slant cultures from spleen, negative February 12

February 12, seventh day *No 2, Series III A* (leukocytes) died of septicemia (?) Large brawny infiltration at site of inoculation in right groin, beginning suppuration, spleen large Smears showed no tubercle bacilli or other organisms in pus from groin

February 13, eighth day *No 4, Series II A* died of septicemia Right inguinal nodes normal (smears negative to tubercle bacilli), spleen and liver large and soft, hemorrhagic pericarditis, pleuritis exudativa Infection probably antedated tubercle bacilli inoculation

February 20, fifteenth day Remaining animals were examined and the lymph-nodes palpated All saline emulsion controls—I A, II A, III A and B were normal I B, C, D, and II B serum animals showed suspicious beginning hyperplasia

March 5, twenty eighth day All examined and distinct enlargement of regional lymph nodes found in all serum and leukocyte animals Controls II A Nos 1, 2 and 3 were questionable

March 9, thirty second day No 4, Series I A control died of chronic septicemia, lymph nodes normal, smears from inguinal and bronchial nodes tubercle bacilli negative, liver large, pale (fatty?), lungs show consolidation of cranial and part of caudal lobes, pregnant (3) near term (This animal was similar to several other old guinea-pigs that were found to have recovered partially from pneumonic septicemia)

March 16, thirty ninth day Seventeen animals were killed by puncture for comparison One of Series I A, all of the Series I B, C and D and all of III A, B and C

The controls in normal saline were all found normal No tubercle bacilli were present in smears from the inguinal nodes

The four guinea pigs Series I B—(Serum 5) were all uniformly diseased with tuberculosis Large caseous inguinal nodes, beginning caseation in iliac, retro

peritoneal, bronchial and cervical chains, "hobnail" spleens with slight caseation, liver also slightly caseated, small isolated tubercles in the lungs

Four of Series I C and three of I D were practically the same as the I B animals, except that the caseation and infiltration of lymph nodes were less marked (Pig 4, I D was not recorded)

The three from Series III A and B (leukocytes) were also practically the same as the serum animals

March 18, forty-first day The remaining Series II A (saline control) and II B guinea-pigs were killed They all showed slightly enlarged inguinal nodes and a few cheesy foci which contained degenerated tubercle bacilli There were no signs of disease beyond inguinal nodes except that in one the iliac nodes were involved

Series II B (Serum 5) Well-marked involvement of inguinal and iliac nodes with slight caseation in all, moderately enlarged spleen and occasional caseous foci in livers, lungs normal, smears from inguinal and iliac nodes reveal well-stained tubercle bacilli

Two fresh guinea-pigs were inoculated from the iliac nodes of No 4, II B and the spleen of No 1 II B to note any possible increased virulence from passage after treatment by serum from Cow 5 They were both killed July 12, four months later, but were found normal One of the first controls Pig 2, I A (saline emulsion), was also killed at the same time (after five months) and found to have chronic tuberculosis in lymph nodes, spleen and lungs The other, Pig 1, I A was lost

Results—This experiment confirmed the results of the previous ones as to the favoring effect of immunized cow-serum on the infective power of tubercle bacilli, whether of virulent or low-virulent stocks

The difference between the effect of active and inactive serums, while less marked than might be expected, was distinct, as was that between the immunized and normal serums No appreciable effect was produced by the leukocytes that could not be accounted for by the influence of the plasma There was at least no evidence of bactericidal action The weakening influence of normal saline was again demonstrated, most of the controls being free from macroscopic disease when killed No increase of virulence could be demonstrated in the culture R1 in the second passage as a result of serum treatment

Discussion

The cause of the paradoxical increase of infectivity of the serum-sensitized bacilli was in itself a problem of importance and led us into further experiments to discover the cause, if possible On the one hand we had found no multiplication of the bacilli *in vitro* and no morphologic changes On the other hand, we knew that the bacilli were agglutinated by the serum in large clumps It was conceivable that they were protected, as it were, with a substance which was inimical to their growth only in the body of the homologous animal, namely, the immunized cow Unfortunately we did not have the facilities for the use of more calves Hence we could not complete the comparison between inoculations of plain and sensitized bacilli We therefore tried corneal inoculations in rabbits, hoping by direct inspection to observe the course of infection from day to day and thereby discover the cause of the differences

Corneal Inoculations in Rabbits

EXPERIMENT 237 E—March 18, 1907 Bled Cows 5, 15 and 16, seven days after injections of tubercle bacilli residue into the first two animals

March 19 Serum separated Agglutination titer Serum 5, 1 to 100 +, Serum 15, 1 to 100 ++, Serum 16, 1 to 33 to 50

An emulsion of virulent human tubercle H38xx, serum slant from March 7, 1907, good growth, was rubbed in 5 c c normal saline, centrifuged ten minutes and filtered twice through sterilized paper The emulsion was faintly opalescent and when diluted once with equal quantities of saline gave smears with 3 to 5 tubercle bacilli to a field and clumps seldom noted The following mixtures were made

A 0 5 c c emulsion tubercle bacilli + 5 c c active Cow Serum 5
B 0 5 c c emulsion tubercle bacilli + 5 c c active Cow Serum 15
C 0 5 c c emulsion tubercle bacilli + 5 c c active Cow Serum 16
D 0 5 c c emulsion tubercle bacilli + 5 c c broth, 2 5 per cent glycerin
E 0 5 c c emulsion tubercle bacilli + 5 c c hydrated chloral 1 5 per cent solution
F 0 5 c c emulsion tubercle bacilli + 5 c c normal saline solution

All incubated twenty-four hours at 38 C (100 4 F) and centrifuged, pipetted + 1 c c H_2O to each tube and transferred to small pointed tubes

Again centrifuged and sediment used by smearing it on a corneal knife for inoculation Three rabbits were used for each series, A, B, C and D Three were used for E and F in the right and left eyes, respectively The periphery of the corneae near the limbus was split under cocain to the width of 2 mm for the inoculation One animal of each series was killed after six hours and another after seven days for microscopic study (which was later abandoned for other work)

The eyes of the remaining rabbits were watched daily and showed characteristic differences A general description will suffice to show them During the first week the earliest to exhibit beginning disease was Series A (serum from Cow 5) Series B was practically the same, while C, D, E and F were progressively less, or had no signs whatever, as in E and F

During the second week metastatic tubercles began to show in the corneae of A, and a well developed focus in B and C as well In the third week subsidence of the series A, B and C was noted, while a gradual beginning disease was observed for the first time in D, E and F

April 16, 1907 Forty seventh day All killed Relations remain unchanged A, B and C have progressed slowly, A shows softening in each focus, B is still quite inflamed, metastases well marked, C is moderately inflamed but less than A and B, D has very slight inflammation remaining, E none, F progressive

Necropsies revealed no internal disease except a few coccidia in two rabbits

Result—A confirmation of previous experiments on guinea pigs in that the sensitized bacilli showed an accelerated infection

The slight difference between the course of the disease in A, B and C in the rabbits' corneae raised the question anew whether or not there was any specific influence of the immunized serums on the course of infection

The following experiment was made to test this point in part, though the result was of no great importance

EXPERIMENT 237 F—April 3, 1907 *Corneal inoculations in rabbits*—Five rabbits were each inoculated in the cornea of one eye with twice the dose of twenty four hour sensitized bacilli with control serum than were inoculated in the other eye with Serum 5 After thirty days the control eyes showed more disease than the others So far as this result could be applied to the question, the number of tubercle bacilli required to infect the eyes to an equal degree was less than twice the number treated with the immunized serum

Cow 5 had no immunizing injections after March 11, 1907, until the following November During July, four months after the last injection

of tubercle bacilli residue, the serum was again tested in comparison with Control 16 by another experiment on guinea-pigs

EXPERIMENT 237 G—July 27, 1907 *Guinea-pig inoculations* Cows 5 and 16 bled

July 29 Serum separated and mixed with an emulsion of virulent tubercle bacilli H39 slant-culture from June 12, 1907, containing four to five single bacilli to a field

1 Took 0 5 c c normal saline emulsion and 3 c c active serum 5
2 Took 0 5 c c normal saline emulsion and 3 c c active serum 16
3 Took 0 5 c c normal saline emulsion and 3 c c inactive serum 16

All incubated twenty-four hours then to each tube 10 c c normal saline was added and centrifuged without stirring The sediments were washed twice with 1 c c normal saline and finally diluted with 2 c c normal saline for inoculation No contaminations

Three lots each of eight guinea-pigs, were given 0 25 c c in the right groin They were all killed on the forty-fourth day and the disease was fairly uniform in all They showed enlarged and caseous regional lymph nodes, spleen, and a few foci in the liver

Result —Probably little difference in the course of infection, but possibly the dose of tubercle bacilli was too large to indicate slight differences Long cessation of treatment of the immunized cow may account for similarity to the control

The results in the last experiment were confirmed by another eye inoculation nine months after the last treatment of Cow 5

EXPERIMENT 237 H—Nov 11, 1907 *Corneal inoculations in rabbits* Serums of Cows 5 and 16 bled Nov 9 1907 Four rabbits inoculated as before with twenty-four-hour sensitized bacilli H39 on opposite eyes

Result —After frequent observations for thirty five days no differences were observed in the course of infection, which was gradual and mild throughout

The injections of tubercle bacilli residue into Cow 5 were resumed Nov 9, 1907, after a negative tuberculin test of 0 5 c c O T The doses were increased every three or four days from 5 c c to 35 c c December 20

Another corneal inoculation was undertaken ten days later with the same technic previously described but using very few bacilli

EXPERIMENT 237 I—Dec 31, 1907 *Corneal inoculations in rabbits*, with serums of Cows 5 and 16 bled December 30 Both agglutinated 1 to 50 Emulsion of H39 sensitized twenty four hours and opposite eyes of ten rabbits were inoculated with a standard loopful of the respective sediments Smears of one loop of each showed clumps of two to five bacilli in a field Serum cultures were also made but revealed no contaminations Careful daily observations on the course of infection were recorded The eyes of the rabbit inoculated with Serum 5 began to show inflammation first, twice as many having redness and conjunctival hyperemia in the region of the inoculation up to the tenth day In the controls the redness was slight in seven eyes on the tenth day, while it was marked in the eyes of all but one of the rabbits inoculated with Serum 5 Seven of the latter also had distinct focal infiltration of leukocytes, but none of the controls showed it until after the tenth day

After twenty-six days five of the Serum 5 eyes had a larger focus, pannus, or beginning ulcer Only one of the controls was more diseased than its opposite when the experiment was terminated, the animals being killed by bleeding and puncture

Result.—Clearly an acceleration of tuberculous infection by the inoculation of bacilli sensitized with specific serum

The method here employed was more satisfactory in that a daily ocular inspection of the progressing disease was possible and the comparisons could be made on the same animal. The human bacillus having mild infectivity for the rabbit restricted the disease to the superior segment of the cornea and the animals betrayed discomfort only during the onset of the infection.

Period of Interrupted Immunization, Cow 5

Another year passed, during which time Cow 5 was under treatment with tubercle bacilli residue until June 11, 1908. Except for an experiment in January, 1908 (to be mentioned later), no further test of the serum was made until six months after the last treatment, when it was determined to repeat the corneal inoculation method to see if there was again a loss of specific effect.

EXPERIMENT 269.—Dec. 22, 1908. Six rabbits were inoculated with sensitized bacilli from Serums 5 and 16, using quantities of culture and technic as nearly identical with the previous test as was possible. The result after thirty days showed no perceptible difference between the eyes as to the course of disease. This confirmed our observation in 1907 that no specific effect was obtainable from Serum 5 so long after cessation of the injections of residue.

INCREASED PROTECTIVE INFLUENCE OF SENSITIZED TUBERCLE BACILLI AGAINST SUBSEQUENT INFECTION IN GUINEA-PIGS

In consideration of the enhanced infective power acquired by our weak virulent human culture R1 when sensitized by Serum 5 (Experiment 237 C2) it was of interest to observe what effect on subsequent virulent reinfection it would have. As in former experiments the R1 culture had shown a marked protection against reinfection with virulent inoculation, it was possible to expect it to be increased, or, on the other hand, it might lessen the resistance of the animals.

Cow 5 was continued under treatment from November, 1907, to June, 1908.

EXPERIMENT 263.—Jan. 8, 1908. Bled Cows 16 and 5 eight days after the last injection of 25 c.c. tubercle bacillus residue.

January 9. Serums centrifuged and added to emulsion of weak-virulent human culture R1 broth from Dec. 17, 1907, fresh growth. Took 0.030 gm. (moist) to 15 c.c. normal saline, centrifuged twenty-five minutes, 5 c.c. supernatant filtered through sterile paper and cotton several times and diluted to 10 c.c. Smears gave 5 or 6 tubercle bacilli per field.

A 1.8 c.c. emulsion + 4 c.c. Serum 5 (active)
B 1.8 c.c. emulsion + 4 c.c. Serum 16 (active)
C 1.8 c.c. emulsion + 4 c.c. culture broth

All incubated twenty-four hours, centrifuged A and B forty minutes, pipeted and replaced by 5.8 c.c. culture broth to each, mixed all tubes thoroughly. Inoculated thirty guinea-pigs, ten for each preparation, with 0.1 c.c. in the left groin subcutaneously. Ten other pigs were selected for control to

the virulent inoculation They were then kept for three months before reinoculation

February 10, thirty second day Palpated and weighed Series A, Pigs 3 and 7, died emaciated February 4, slightly enlarged nodes in left groin, no others have palpable enlarged nodes, all but one losing weight

Series B, Pigs 1 and 7 died in January, chronic pneumonia No signs of tuberculosis no enlarged nodes, all but three gaining weight

Series C, no deaths, no palpable enlarged nodes all have lost weight

Series D (controls) One died of chronic pneumonia February 17, all but three losing weight

February 17 and 18 Pig 4A and 4C died of chronic pneumonia, no other disease evident

March 5 Pig 10A died emaciated no disease except left inguinal nodes larger than normal

March 6 Pigs 1 and 4D died the former only emaciated the latter with large submaxillary abscess

March 19 Pigs 5 and 6C, 6 and 11D were taken for another experiment

March 20, sixtieth day The twenty six remaining animals were each inoculated in the right groin with 0 3 c c centrifuged, filtered emulsion human tubercle bacilli H37 broth culture March 3, 1908 Smears of emulsion show from 5 to 6 per field Practically all the guinea-pigs had gained weight except Pig 6 of Series A and Pigs 3 and 6 of Series B

April 5 Pig 8A died emaciated, no visible disease both inguinal chains normal

April 29 All killed for comparison except Pig 7A and Pig 10B (suckling young)

Of the four, Series A none had any trace of disease in the left inguinal nodes, but showed some tuberculous involvement in the right, smears were tubercle bacilli positive from the last, but negative in the other, and also from the iliac nodes and spleens of all this series otherwise normal

Of the six of Series B, two had both inguinal nodes involved all had right two with abscesses in the left, two in the right, one with iliac and the spleens of three were suspicious though in two smears were negative, otherwise normal

Of the seven of Series C, there was a close resemblance to Series B in most of them, only in one the left inguinal node being enlarged

Of the six controls, D, all had extensive uniformly caseous nodes, spleen and liver, the latter in the first stages The lungs were not diseased

Pig 10B died emaciated a month later with no visible disease Pig 7A lived until Feb 19, 1909, when it succumbed to advanced fibroid tuberculosis presumably from the virulent infection

Result—There was a slight difference in favor of the immune serum sensitized animals as to the extent of the infection, it being less than any of the controls None showed the amount of disease found in the normal serum controls, though the latter had hardly reached beyond the inguinal-iliac chain of lymph nodes

There was an unusually large mortality at first in this experiment from chronic pneumonia, which detracted from its value in point of numbers The result was, however, suggestive of a specific influence in aiding the protective inoculation

Further Immunization of Cow 5

In consideration of the low agglutination titer and complement deviation that we had obtained with Serum 5, it was determined to try larger inoculations of human bacilli followed by tubercle bacillus residue before abandoning the search for bactericidal properties in the serum

The dangers of intravenous inoculations containing clumps of bacilli from emboli in the brain and other organs necessitated caution

In addition to this danger, which in one of our own calves and those of other experimenters had led to tuberculous abscesses in joints, there was danger of anaphylactic shock, which at that time was well recognized as such, though formerly misinterpreted By sedimentation and dilution we hoped to avoid the first danger, and by moderate dosage, the second In this we were successful, and during the next two years Cow 5 received four more inoculations of living, virulent human bacilli H37 besides intervening treatment with tubercle bacilli residue and other tuberculins

The dates of inoculations were as follows

Dec 29, 1908 Third inoculation, 10 c c centrifuged emulsion, estimated (by deducting sediment) 0 060 gm

August 5, 1910 Fourth inoculation, 25 c c centrifuged emulsion, estimated as previously, 0 170 gm

Sept 20, 1910 Fifth inoculation, 25 c c centrifuged emulsion, same estimation, 0 175 gm

Dec 30, 1910 Sixth inoculation, 25 c c centrifuged emulsion, estimated as before, 0 130 gm

Fever reactions from 103 to 106 F followed each dose within six hours, but subsided on the next day The cow remained in fine flesh and condition throughout the further treatment, yet no reactions resulted from subcutaneous tuberculin injections of various kinds Feb 1, 1911, 1 c c O T diluted was given intravenously There was respiratory embarrassment for ten minutes followed by a fever to 105 6 in six hours Feb 7, 1911, 1 5 c c O T was given in the same way Marked difficulty and acceleration of respiration followed, with dizziness and a temperature of 104 5 in six hours

The symptoms of anaphylaxis were too well marked to permit further intravenous injections Further subcutaneous injections of W E and T O were given, but produced no higher agglutination titer than 1 to 100 A slight precipitation reaction was obtainable The complement deviation was also found no lower in many tests with the same stock antigen The titer during 1910 and 1911 was 0 02 c c serum to completely absorb complement with 0 1 c c T R emulsion (0 001 gm dry to 1 c c) Considering the unpromising outlook for a higher grade of antibodies the immunization was discontinued April 27, 1911[13] The animal was still in perfect condition when slaughtered in December, 1912 Very unfortunately no post mortem examination was feasible, as she was kept at a distance from the town

Final Experiments to Test Bactericidal Effect of Serum, Cow 5

EXPERIMENT 206 A.—June 23, 1909 *Subcutaneous Inoculations* Six months after the third reinoculation, during which time continuous treatment with tubercle bacilli residue was carried on Bled Cows 5 and 16

July 27 Incubated with emulsion virulent human culture H41 (prepared as in previous experiments) four days without change Centrifuged, inoculated subcutaneously into five pigs for each test in right inguinal region Serum 5 animals died sooner than those of Serum 16, the average being 36 2 days as compared with 55 2 days for the controls

The Serum 5 animals emaciated and revealed disease in the inguinal and iliac nodes with caseation or beginning abscess One survived three months

13 It must be confessed that we dropped this work at this point because other things seemed more important, and it was impossible to keep large animals near the laboratory with our limited facilities

and had an abscess in the groin with spleen and lungs involved The controls that survived longer had more disease and three showed caseous nodes and foci in the spleen

Result—No specific effect of Serum 5 was demonstrated in this experiment unless the shorter average lifetime of the Serum 5 animals may be ascribed to some toxic action produced by the serum acting on the tubercle bacilli and which was liberated in the animals The animals were in poor condition at any rate

A final attempt to show a bactericidal influence of Serum 5 was made a month after the sixth and last inoculation, and a week after a tuberculin reaction on Feb 1, 1911

EXPERIMENT 206 B—Feb 7, 1911 *Intracutaneous inoculations* A different method was tried in this experiment by comparing intracutaneous inoculations made on the same animal simultaneously A new normal cow was used for control serum, and both active and inactive serums used, together with a broth control Opposite sides of six guinea-pigs were shaven and the inoculations made after three days' incubation with human tubercle bacilli H37 The inoculations were made sufficiently far apart to cause any marked difference in the reactions to be noticeable The infections were practically the same up to the nineteenth day, when all but the broth controls began to show caseous centers The Serum 5 (active) seemed more inclined to ulcerate than the rest yet the infiltration and redness were practically alike At this time no lymph nodes showed enlargement As no certain differences were assured the animals were soon killed

Result—No evidence of specific action from Serum 5 in this experiment

DISCUSSION

The general effect noted throughout the foregoing experiments was unfavorable so far as any practical value of the immunized cow-serum in the therapy of tuberculosis

The sensitized living bacilli which we hoped might be weakened by the serum were in fact rather more infective and therefore less available for vaccines

The phenomenon of increased infectivity was associated with serums from the cow under active immunization with T R During resting periods the serum lost its property for the time, but soon regained it when the injections of T R were resumed The antibody reactions were never strong, and the agglutination was the most delicate and pronounced of any The serum titer was, notwithstanding, often no higher than that from a normal cow, so that it was difficult to understand that the difference in infectivity was due to the greater agglutination Considering the claim that serum-sensitized B E tuberculin is more easily absorbed than the plain emulsion, one may account for the increased virulence produced in guinea-pigs and rabbits by the bacteriotropic effect of the serum on living bacilli as well as by the agglutination

It may be that specific agglutinins and bacteriotropins excite a more active phagocytosis under these conditions The leukocytes ingest large clumps of agglutinated bacilli which they are unable to dispose of In the non-sensitized bacilli they find them in singles and can weaken

or kill them more readily As a working theory the preceding explanation, partly mechanical as well as biological, may account for our results In many other pathogenic organisms one may note rapid lysis under the influence of antiserums or in similarly sensitized bacteria when inoculated Even were an active lysin present in our cow-serums the difficult solubility of the tubercle bacilli undoubtedly masks it

It has in fact been suggested that by prolonged digestion of living tubercle bacilli in active immune serums (as in our experiments) the weaker bacilli are devitalized, if not dissolved, leaving only the most vigorous and resistant ones The latter when inoculated, being clumped together, are able to survive the cellular digestion longer In these ways one may explain the seeming paradox in our results and the causes for the earlier reaction and further spread of the disease in the inoculated animals

Intracellular digestion of tubercle bacilli is slow when observed in the peritoneal exudate of previously infected animals It is much slower in control animals and few observers have claimed to dissolve them at all in immune serum *in vitro* The peculiarity of the wax in tubercle bacilli places this organism in a class by itself as unusually resistant against lysis Under the most favorable conditions for the lysis of tubercle bacilli a few often escape and may produce an infection, hence the difficulty of experimental demonstration of the mechanism

Some consideration should be given to the possible influence of the cow-serum as such in our experiments In most cases it was washed out of the prepared bacilli and only the antibody fixed to the bacilli could come into the question There is no reason to think that it caused any aggravation of the infection, except through the anchored antibody It is true that other observers (Calmette and Guérin[14]) have found that immunized cow-serum aggravated the disease in guinea-pigs, though in a recent tentative experiment Rothe and Bierbaum[15] on two guinea-pigs thought that 0 1 to 0 2 c c immunized cow-serum mixed with tubercle bacilli twenty hours weakened the infection Immunized cow-, horse- and mule-serum were found by Ruppel and Rickman[16] to restrain infection in guinea-pigs by injections of 5 c c, though it is not stated which serum was used, nor have further details been published

Nearly all of the earlier experiments were with repeated injections of horse-serum or that of other immunized animals Bâbes and Proca,[17]

14 Calmette and Guérin Ann d l'Inst Pasteur, 1911, xxv, 626

15 Rothe, E, and Bierbaum, K Veroffentl d Robert Koch Stiftung 1913, Nos 8, 9, p 169

16 Ruppel and Rickman Ztschr f Immunitatsforsch, 1910, vi, 383

17 Babes, V, and Proca G Ztschr f Hyg u Infectionskrankh, 1896, xxiii, No 3, p 331

Karwacki [18] They were usually harmful because of the anaphylactic sensitization produced, which at the time was not understood

In all these experiments with antituberculous serums it must also be remembered that an anaphylactic antibody to tuberculo-protein must frequently be present in the serum This might be a source of slow poisoning to a tuberculous animal by acting on the bacilli and setting free small amounts of anaphylatoxin It is conceivable that the early reaction and greater irritation of the rabbit cornea seen in our experiments with immune-serum-sensitized bacilli were due to this cause The probability of this is lessened by the fact that the poison would have been equally strong, if not stronger, from the bacilli sensitized with inactivated than with the active serum

To recapitulate the conclusions of most importance, the following summary may be presented

SUMMARY

1 The foregoing experiments relate to the properties of the serum of a cow repeatedly immunized against tuberculosis during a period of ten years

2 Six intravenous inoculations of living human cultures of tubercle bacilli, both of virulent and weak-virulent infectivity, besides numerous injections of all forms of tuberculin, were given to this cow

3 Specific agglutinin, precipitin, opsonin, and complement-deviating antibody were demonstrated in varying degree in the serum but never reached a high content

4 The presence of a bacteriolysin was not demonstrated, neither was a bacillicidal effect recognized either in the serum or leukocytes

5 Living human tubercle bacilli "sensitized" with the immune serum showed increased infective power in guinea-pigs and rabbits as compared with normal cow-serum

6 The increased infective power was manifested by earlier and more marked reactions or inflammation following inoculation, and a wider spread of the disease than in controls

7 This acceleration of infection was not manifest unless the cow had recently received injections of pulverized bacillus residue, but the immune-serum-agglutinin titer was not always greater than the normal cow serum

8 To explain the apparent paradoxical action of the immune serum, it is suggested that the strongly agglutinated bacilli were protected from injury by the leukocytes of the inoculated animal by reason of the clumping The bacilli were presumably phagocyted in large masses but more difficult of digestion for that reason It may also be suggested that the

18 Karwacki, L Ztschr f Tuberk, 1906, viii, 52 59

bacilli laden with antibody (opsonin) were more rapidly phagocyted and scattered, but owing to the resistant wax were not digested in sufficient numbers ,

9 A "sensitized" *living* tubercle-bacillus vaccine is not safe or practicable when prepared from immune bovine serums, considering the above-mentioned results

My heartiest acknowledgements are due, especially to Dr Joseph L Nichols, Dr J Woods Price, and the late Dr Albert H Allen, who assisted in these studies

The expense of this work was borne by Mrs A A Anderson of New York City, to whom we also owe our thanks

AN EXPERIMENTAL STUDY OF INTESTINAL OBSTRUCTION [*]

JOHN A HARTWELL, M D, J P HOGUET, M D
AND
FENWICK BEEKMAN, M D
NEW YORK

Intestinal obstruction in man, if unrelieved, speedily causes death The fatal outcome is too rapid to be the result of starvation, and three general theories have been advanced to explain it

1 A disorder of the nervous mechanism controlling the cardiac and vasomotor systems

2 A bacterial infection of the organism by the passage outward of bacteria from the intestinal lumen

3 An intoxication from poisonous substances imprisoned in the intestine orally to the obstruction

Two modifications of the last theory have recently come into prominence The first was originally advanced by Vidal,[1] and more recently elaborated by Maury Draper[2] (formerly Draper Maury), in which the presence of a substance in the upper intestinal tract was believed to exist, which unless neutralized by substances elaborated lower down, entered the system as a poison

The second modification is advanced by Whipple, Stone and Bernheim[3] These workers believe that stagnation of the duodenal contents results in the formation of a severe poison, which readily enters the system, and produces death This poison may be obtained from the mucosal cells of the duodenum as well as from the duodenal contents

THE THEORY OF A NERVOUS DISORDER

In favor of this theory, clinical evidence has been adduced The tachycardia, the low blood-pressure, the profound collapse of the patient,

* From the Department of Surgery of the Cornell University Medical College
* Submitted for publication March 3, 1914

* Preliminary reports on the work here detailed have appeared in Am Jour Med Sc, March, 1912, p 357, Jour Am Med Assn, 1912, lix, 82, Jour Exper Med, 1913, xviii, No 2, p 139 These reports are freely abstracted in the present article in order to present the subject in its entirety

1 Vidal Rev de chir, 1900, xxvi, 521, Cong Franc de chir, 1905, xxviii, 1237

2 Draper, J W Experimental Intestinal Obstruction, Jour Am Med Assn, 1911, lvii, 1338, John Hopkins Hosp Bull, 1909, xx, 184, Am Jour Med Sc, 1909, cxxxvii, 725, Death in Acute Intestinal Obstruction and Kindred Conditions Is Due to Physiologic Disturbance, Jour Am Med Assn, 1910, liv, 5

3 Whipple, Stone and Bernheim Jour Exper Med, 1913, xvii, No 3, p 286

and the wide dilatation of the splanchnic blood-vessels, all point to a loss of activity on the part of the medullary nerve-centers The early writers saw in these the results of a reflex disturbance from stimuli acting on the nerve endings in the intestinal wall It is undoubtedly true, however, that exactly this train of events may arise from a toxemia or a bacteriemia without the intervention of any afferent nerve impulses

Recently, Braun and Boruttau[4] have modified this theory somewhat, and assign the above-described symptoms to a disturbance of the circulation resulting from interference, chiefly mechanical, with the inherent nerve plexuses in the intestinal wall They see a parallel between a gradual bleeding to death and the death in intestinal obstruction The loss of body fluids resulting from failure of absorption, the enormous outflow of fluid into the intestine, the persistent vomiting, and the stagnation of blood in the splanchnic area lead to an anemia of the brain-centers which progresses till these centers cease their activity, and death results They experimented most exhaustively on rabbits to support this view, but the evidence is all indirect, and seems to us not to amount to a proof of the theory We are unable to find in the literature any convincing experimental work that the nervous system is primarily at fault Certainly, as death approaches, there is a profound disturbance of the nervous control of the heart, blood-vessels and respiration, but this, we believe, is in no sense a reflex disturbance, nor the result of an anemia, *per se,* of the nerve-centers It differs in no way from the last stages of any severe sickness which is progressing toward a fatal issue The loss of body fluids is a matter of the utmost importance, but not because of its effect in producing a brain anemia

THE THEORY OF BACTERIAL INFECTION

The infection theory has much more to support it The intestinal lumen is the normal habitat of innumerable micro-organisms which are potentially pathogenic When an obstruction exists, these no longer have their normal outlet per anum They are dammed up in the intestine under favorable conditions for their growth (McClure[5]) The damage to the intestinal mucosa may allow their passage outward, either into the blood-stream, lymphatics, or directly into the peritoneal cavity Proof of this possibility is seen clinically in the presence of a peritonitis as a complication of intestinal obstruction without perforation, also in the frequent finding of colon bacillus in the blood as a pathogenic micro-organism, under very varied conditions of intestinal disturbances less severe than an obstruction, and in the bacteriemia from the *Bacillus typhosus* when the intestine is ulcerated by the action of that organism

4 Braun and Boruttau Deutsch Ztschr f Chir, 1908, xcvi, 544

5 McClure, R D An Experimental Study of Internal Obstruction, Jour Am Med Assn, 1907, xlix, 1003

Von Khantz,[6] experimenting on rabbits in a low intestinal obstruction, often found a bacterial invasion of the blood and the peritoneum, but never the former without the latter Borszesky and von Genersich,[7] on the other hand, found a blood infection with no invasion of the peritoneum There can be no doubt that a bacterial invasion of both the peritoneum and the blood may take place in intestinal obstruction, but it is not an essential accompaniment It occurs late as a complication, often a terminal one Both clinically and experimentally it will result most often when the obstruction is complicated by a strangulation Without this complication, intestinal obstruction will kill with no passage of bacteria beyond the intestinal wall.

Tables 1 and 2 give the results of our experiments bearing on this point In the first series the obstruction was produced by dividing the intestine from 10 to 30 cm below the pylorus, and closing the ends by inversion It will be noted that in all these cases some micro-organisms were found in the organs examined This, we believe, was due to the fact that all the examinations were made several hours post mortem, because all the animals died during the night, and possibly to a local soiling at the site of operation In the second series, however, the obstruction was produced by the clamp described below, and the animals were either killed when it was believed they were too sick to live over night, and immediately examined, or examined very soon after death occurred from the obstruction itself This series of seven cases furnishes irrefutable proof that death results from an obstruction of the upper intestine in the dog, without any invasion of the peritoneum, blood, liver or spleen by bacteria, which are demonstrable by the methods employed The methods in the two series were exactly similar and their efficiency in detecting organisms is amply demonstrated in the first The cultures were taken at autopsy on slant agar or Loeffler blood-serum, or both Usually four tubes sometimes only three of each, were inoculated from each tissue, and grown under both aerobic and anaerobic conditions at incubator temperature For the anaerobes the Buchner method or the Cornell modification of it was used The tubes were examined at the end of one, two and three days All the cultures were made in the sterile room of the Cornell bacteriology laboratory This consists of a closed cabinet, ventilated through a bacteria-proof filter, in the roof of which is suspended an ordinary revolving lawn-sprinkler The water is turned through this for fifteen or twenty minutes prior to taking the cultures Thus the air is mechanically sterilized by washing out the micro-organisms All the needed apparatus for taking the cultures and smears is assembled before beginning the inoculations,

6 Von Khantz Arch f klin Chir, 1909, xxxviii, 412
7 Borszesky and von Genersich Beitr z klin Chir, 1902, xxxvi, 448

and the air-tight door is kept closed until all cultures are completed The technic in making the inoculations is that usually employed in obtaining cultures from post-mortem material The efficiency of the room and the method is demonstrated by the fact that only three air contaminations were found in several hundred tubes

In some of the later cases of the second series no cultures were taken from the spleen, since it was noted that this organ never showed infection when the liver and peritoneum were without infection Often, too, the spleen cultures gave no growth when the other organs were actively infected A summary of this series shows that the femoral blood was cultured seven times, and always was without growth, the liver was cultured six times, and showed one colony of Gram-positive cocci, in one tube of one case, probably an air contamination, the results from the peritoneal cultures were exactly the same as from the liver, the spleen was cultured only twice, with negative findings both times, the jejunal or duodenal contents above the clamp were cultured seven times, and all tubes showed an abundant growth of various micro-organisms

TABLE 1.—CULTURES FROM ORGANS OF ANIMALS WITH INTESTINAL OBSTRUCTIONS, SERIES 1

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
12	Spleen	2 aerobic 3 anaerobic	None	1 aerobic 2 anaerobic	Morphology and growth characteristic of colon bacillus on usual differential mediums	Intestine sectioned and closed 34 cm distal to pylorus Lived 80 hours Cultures taken 10 hours post mortem
	Liver	2 aerobic 4 anaerobic	All	All	(1) Gram + coccus (2) Bacillus identified as colon on differential mediums (3) Also very many colonies of a large Gram + anaerobic bacillus with morphology of *Bacillus aerogenes capsulatus*	
	Jejunum	2 aerobic 4 anaerobic	All	All	(1) Gram + coccus (2) Large anaerobic Gram + bacillus (3) Gram — bacillus of two kinds, first long and slender, second, short and plump Both grew in anaerobic tubes, while the former only grew in aerobic tubes	
13	Femoral blood	4 each	None	None		Intestine sectioned 36 cm from pylorus and ends closed Autopsy about 13 hours post mortem
	Peritoneum	4 each	All	None	(1) Gram + and (2) Gram — coccus	
	Spleen	4 aerobic 3 anaerobic	1 aerobic	None	Gram + coccus Same organism as in peritoneum	
	Liver	4 each	None	None		
	Jejunum	4 each	All	All	Same as in Case 12	Cultures taken from jejunum when it was sectioned at operation gave the same results as at autopsy

TABLE 1—CONTINUED

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
14	Jejunum at operation	2 aerobic 2 anaerobic	All	All	(1) Gram — bacillus (2) Gram + coccus (3) Large Gram + bacillus	Intestine sutured after section 31 cm from pylorus Autopsy 36 hours post mortem
	Jejunum at autopsy	4 each	All	All	(1) Anaerobic gas forming Gram — bacillus (2) Gram + bacillus (3) Gram + coccus	
	Femoral blood	4 each	2 aerobic	All	(1) Gram + coccus ferments lactose and glucose, without gas no change in neutral red and saffranin (2) Large anaerobic Gram + bacillus with morphology of aerogenes capsulatus bacillus	
	Peritoneum	2 each	All	All	(1) Gram + coccus same as in femoral blood (2) Gram — bacillus like colon bacillus	
	Spleen	4 each	2 aerobic	2 anaerobic	(1) Gram — coccus (2) Gram — bacillus having the characteristics of colon bacillus	
	Liver	2 anaerobic 4 aerobic	2 anaerobic 3 aerobic	None	(1) Gram + coccus as in peritoneum and femoral blood	
15	Jejunum at operation	4 each	4 anaerobic	4 anaerobic 2 aerobic	Gram — bacillus, mobile same as in peritoneum	Intestine ligated with tape 35 cm from pylorus Autopsy 4 hrs post mortem General peritonitis present
	Jejunum at autopsy	4 each	1 anaerobic	All		
	Peritoneum	4 each	4 anaerobic	All	(1) Gram — bacillus having characteristics of colon bacillus (2) Gram — bacillus intermediate in colon group, changes neutral red and saffranin does not ferment lactose (3) Gram + coccus	
	Femoral blood	4 each	None	None		
	Spleen	4 each	None	None		
	Liver	4 each	None	None		
		4 each				
16	Jejunum at operation			All	Not identified	Bowel sectioned and inverted 18 cm from pylorus Lived about 12 hours Autopsy 12 hours post mortem No peritonitis
	Jejunum at autopsy	4 each	All	All	(1) Short Gram — bacillus (2) Gram + coccus resembling streptococcus (3) Large Gram + bacillus	
	Femoral blood	4 each	None	None		
	Spleen	4 each	None	None		
	Liver	4 each	All	All	Gram — short, stout bacillus mobile (2 strains), resembling colon bacillus (2) Gram + bacillus (3) Gram + coccus	
	Peritoneum	4 each	All	All	(1) Gram + coccus (2) Gram + bacillus (3) Gram — bacillus short and stout, mobile Same as in liver	

TABLE 1—CONTINUED

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
17	Jejunum at autopsy	None taken	None taken			Jejunum clamped 37 cm from pylorus with Crile clamp, which at autopsy was found to have cut through Autopsy 8 hours post mortem, lived about 88 hrs General peritonitis present
	Femoral blood	4 each	None	None		
	Spleen	4 each	1 anaerobic	None	Gram + coccus One colony outside line of inoculation Probable air contamination	
	Liver	4 each	None	None		
	Peritoneum	4 each	4 anaerobic 3 aerobic	4 anaerobic 3 aerobic	(1) Gram — bacillus, having characteristics of coli communis (2) Gram + coccus (3) Gram + bacillus short and fat and growing in long chains	
18	Jejunum	None taken				Jejunum clamped with Crile clamp 20 cm from pylorus Killed with chloroform 91 hrs after operation Clamp cut through, local peritonitis resulting
	Femoral blood	4 each	None	None	(1) Gram — bacillus	
	Peritoneum	4 each	None	All	(2) Gram + bacillus	

TABLE 2—CULTURES FROM ORGANS OF ANIMALS WITH INTESTINAL OBSTRUCTIONS, SERIES 2

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
19	Jejunum	3 aerobic 3 anaerobic	3 anaerobic	3 anaerobic 3 aerobic	(1) Gram — bacillus (2) Gram + bacillus	Intestine occluded with special clamp 18 cm from pylorus Lived 172 hrs after operation Killed with chloroform Autopsy immediately No peritonitis, local abscess around clamp
	Femoral blood	4 aerobic 3 anaerobic	None	None		
	Spleen	4 aerobic 4 anaerobic	None	None		
	Liver	4 aerobic 3 anaerobic	None	None		
	Peritoneum	3 anaerobic 4 aerobic	None	None		
	Abscess around clamp	2 aerobic 2 anaerobic		1 anaerobic 2 aerobic	Gram — bacillus	
20	Jejunum	None taken				Occlusion clamp 16 cm from pylorus Lived 90 hrs Died Autopsy No peritonitis
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Liver	4 anaerobic 4 aerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
	Exudate around clamp	3 aerobic 3 anaerobic	None	None		
21	Jejunum	4 aerobic	4 anaerobic	4 anaerobic	(1) Gram + coccus (2) Gram + bacillus (3) Gram — bacillus	Occlusion clamp on jejunum 16 cm from pylorus Dog lived 58 hrs Autopsy 8 hrs post mortem Peritoneum clean
	Femoral blood	4 anaerobic 4 aerobic 4 anaerobic	4 aerobic None	4 aerobic None		
	Spleen	4 aerobic 4 anaerobic	None	None		
	Liver	4 aerobic 4 anaerobic	None	None		
	Peritoneum	3 aerobic 4 anaerobic	1 aerobic		Gram + coccus One colony only, probably air contamination	

TABLE 2—CONTINUED

Case	Organs	Cultures Taken	Tubes Showing Cocci	Tubes Showing Bacilli	Characteristics	Remarks
22	Jejunum	3 aerobic 3 anaerobic	2 aerobic 1 anaerobic		Gram + cocci	Occlusion clamp 12 cm from pylorus Lived 21 days Killed with ether Clamp not absolutely tight. No peritonitis
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Liver	3 aerobic 3 anaerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
23	Jejunum Liver	None taken None taken				Occlusion clamp 12 cm from pylorus Lived 210 hrs Killed with cyanid and autopsied immediately No peritonitis Clamp tight.
	Femoral blood	3 aerobic 4 anaerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
24	Jejunum	2 aerobic 2 anaerobic	None	2 aerobic 2 anaerobic	Gram + and — bacilli	Occlusion clamp 16 cm from pylorus Lived 205 hrs Autopsied 2 hrs post mortem No peritonitis Small abscess around clamp was tight
	Femoral blood	3 aerobic 4 anaerobic	None	None		
	Liver	4 aerobic 4 anaerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
	Clamp abscess	2 aerobic 2 anaerobic	2 anaerobic 2 aerobic	None	Pure growth of Gram + coccus	Occlusion clamp 16 cm from pylorus Lived 240 hrs Killed with ether Autopsy immediate No peritonitis
26	Jejunum	4 aerobic 4 anaerobic	None	1 aerobic 4 anaerobic	(1) Small Gram — bacillus (2) Large, long Gram + bacillus	
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Liver	3 aerobic 4 anaerobic	1 aerobic	None	One colony outside inoculation of a Gram + coccus	
	Peritoneum	4 aerobic 4 anaerobic	None	None		
28	Jejunum	2 aerobic 2 anaerobic	2 anaerobic	2 aerobic 2 anaerobic	Gram + and — bacilli and cocci Anaerobic tubes show gas production	Occlusion clamp 11 cm from pylorus Lived 56 hrs Autopsy 10 hrs post mortem Abscess around clamp No peritonitis Clamp tight
	Femoral blood	4 aerobic 4 anaerobic	None	None		
	Liver	4 aerobic 3 anaerobic	None	None		
	Peritoneum	4 aerobic 4 anaerobic	None	None		
	Clamp abscess	2 aerobic 2 anaerobic	None	None		

Case 22, given in Table 2, is not included in the summary because the obstruction was not complete It is worthy of note here, however, that the jejunal tube only showed a growth of a Gram-positive coccus in one-half the tubes inoculated, showing that the long period (twenty-one days) with only sterile water by mouth did much toward sterilizing the upper bowel

It is thus demonstrated that no matter what the rôle of the microorganisms may be within the bowel, or no matter how active they may sometimes become in causing complications by penetration of the bowel into the general system, this is not a necessary factor either in causing

the symptoms of the disease or in determining the fatal outcome The theory, therefore, which holds a bacteriemia as the responsible factor in the death following intestinal obstruction is untenable

THE INTOXICATION THEORY

This theory, on the contrary, has experimental evidence to support it, and no experiments have proved the absence of a toxemia Clairmont and Ranzi[8] demonstrated that the stagnated material above an obstruction, after passage through a Reichel or Pukal filter, was exceedingly toxic when injected into the blood of an animal Kukula[9] found a toxic material in the alcoholic extracts of the intestinal contents in experimental ileus Roger,[10] and Roger and Garnier,[11] went farther and proved that the normal content of both the stomach and the intestine are poisonous when thus injected They did not filter it, but death occurred too early to be the result of an infection The toxicity was only one-third as great when injected into the portal vein as when injected into a peripheral vein They also injected it into a mesenteric artery, against the current, so that it was carried backward and then redistributed through branches to a considerable section of the intestine Here it produced emboli and stopped the absorption, so that large doses were not fatal

Murphy and Vincent[12] found that the material from the obstructed or strangulated intestine was very poisonous when injected into the peritoneal cavity, and that its poisonous properties were destroyed by boiling or passage through a Berkefeld filter They concluded, therefore, that living bacteria are the important factors It does not seem to us that this conclusion is tenable Their experiments only prove that living bacteria, if they obtain entrance into the peritoneum, will kill

Whipple, Stone and Bernheim[3] worked with a material obtained from the doubly occluded duodenum, and determined that it was very poisonous even after being rendered sterile They also found what they believed to be the same poison in the mucosa of this part of the intestine, when one end of it was closed and the other end allowed to drain externally They applied their results to a simple intestinal obstruction, and concluded that death in this condition is caused by a poison arising from the duodenal mucosa Their views are opposed in important particulars to the results obtained by us, and will receive closer study later In general it may be stated that the symptoms aris-

8 Clairmont and Ranzi Arch f klin Chir, 1904, lxxiii, 696
9 Kukula Arch f klin Chir, 1901, lxiii, 773
10 Roger Rev Scient, 1907, xlv, 65 Presse méd, 1911, xix, 1
11 Roger and Garnier Arch de méd expér, July, 1906, Rev de méd, 1906 xxvi 609, Presse méd, 1906, xiv, 325
12 Murphy and Vincent Boston Med and Surg Jour, 1911, clxv, 684

ing from the injection of intestinal contents into the blood, as reported, differ very markedly from those appearing in the obstructed animal, and there are many objections, as pointed out by Guibe,[13] Braun and Boruttau,[4] Korentchevsky,[14] and others, against accepting these experiments as directly bearing on the cause of death in intestinal obstruction The lessened absorption from the obstructed intestine is one objection offered Clairmont and Ranzi[8] found that potassium iodid placed in the bowel above the obstruction was excreted from the kidneys in greater quantities than normal, up to eight or ten hours after the obstruction was produced, the excretion rapidly decreased after that, and came to a stop in about fifteen hours

Braun and Boruttau[4] gave strychnin to their obstructed animals, and from the resulting symptoms they concluded that absorption became markedly reduced from the start On this experiment they base their chief objection to the intoxication theory A more important objection to the use of the evidence of the injection experiments as a proof of the intoxication theory is that many substances injected directly into the circulation are poisonous, whereas, if they have to pass through living cells to get into the blood, their poisonous properties are entirely destroyed Peptone is such a substance, and is a good illustration in this connection The injection of peptone into the blood produces symptoms of poisoning which may end in death The absorption of peptone from the intestine is, on the contrary a normal physiologic process Therefore, the mere presence in the obstructed intestinal tract of substances which act as poisons when injected into the blood is no indication that these substances cause the death resulting from an obstruction, since such substances may be normally found in the intestine in conditions of continued health To accept the view that such a death results from a toxemia it must be conceived either that new poisons are elaborated and absorbed or that an abnormal absorption of the poisons normally takes place

In undertaking our work, after a study of the literature, we tentatively accepted the first hypothesis and planned our experiments to determine by a process of exclusion from what sources conception was forced on us, namely, *that the essential factor in causing the symptoms and death in intestinal obstruction does not lie in the poisons per se, but in the production of lesions which favor their abnormal absorption, and lacking these lesions the animals die only from dehydration, or starvation if dehydration is prevented* We believe our reader will get a clearer understanding of our findings if he will follow, step

13 Guibe Presse méd, 1909, xvii, 233

14 Korentchevsky Roussky Vratsch, 1908, p 1572 (Cited by Guibe, Note 13)

by step, the reasoning and experiments which led to this change of conception in our minds

In searching for the source of the poison we accepted three possibilities (1) foodstuffs or substances derived from them, (2) secretory substances from the alimentary tract and digestive glands, or their derivatives, (3) true bacterial toxins It was our intention to exclude one after the other of these possible sources and study the conditions resulting after an obstruction was produced It is readily seen that there may enter into all of these the result of bacterial activity While, as detailed above, our bacterial studies showed that a bacteriemia is not necesasrily present, they did not exclude either a bacterial toxin or substances developed by virtue of the presence of bacteria in the occluded bowel

EXPERIMENTS TO DETERMINE THE EFFECT OF FOODSTUFFS OR THEIR DERIVATIVES ABOVE THE POINT OF OBSTRUCTION

All animals were fully etherized before the operation No food was given in any case after the operation, unless so stated Water, however, was given freely, unless otherwise noted

EXPERIMENT 3.—A cat which had received no food for ninety six hours before operation Duodenum sectioned and ends closed by inversion 10 cm below pylorus For three days the animal remained in good condition Vomited Fourth day showed weakness and some rigidity of muscles of extremities Just before death had convulsions involving extremities Killed with chloroform ninety six hours after obstruction Autopsy immediate No peritonitis Closure of bowel ends tight Stomach contained small amount of normal appearing gastric content Duodenum empty Neither stomach or duodenum was distended

EXPERIMENT 4.—Fox terrier Fed twenty hours before operation Repetition of Experiment 3, the section being 11 cm distal to the pylorus Except for some weakness and vomiting, seemed like normal dog for four days Died with progressive weakness and vomiting on the seventh day Autopsy No intestinal leakage No peritonitis Stomach slightly distended Duodenum not distended

EXPERIMENT 5.—Bitch Weight 9 8 kg Fed last time forty-eight hours before operation Repetition of Experiment 3 Postoperative course the same Lived one hundred hours Autopsy No intestinal leakage No peritonitis No distention of stomach or duodenum

EXPERIMENT 6.—Bitch Weight 10 85 kg Food withheld for seventy two hours Repetition of Experiment 3, section being 12 cm below pylorus Postoperative course the same Temperature varied from 36 6 to 39 C Lived for fifty five hours Autopsy Same findings as those given above

EXPERIMENT 7.—Dog Weight 19 7 kg Fed last time forty eight hours before operation Same operation 16 cm below pylorus Some peritoneal soiling with intestinal contents Postoperative course same as others, except more acute Temperature as high as 39 9 C Lived only thirty six hours Autopsy yielded same findings as others

EXPERIMENT 8.—Dog Medium weight Fed six hours before operation Same operation 12 cm below pylorus Postoperative course the same Temperature 39 C Lived one hundred and eighteen hours Autopsy Same findings and in addition there were noted three superficial areas in the duodenum which resembled ulcers

EXPERIMENT 9.—Leakage of intestinal contents at closed end of proximal gut.

EXPERIMENT 10.—Same.

EXPERIMENT 11.—Dog. Weight 5.3 kg. Fed twenty-six hours before operation. Same operation 33 cm. below pylorus. Lived only ten to fourteen hours. Autopsy. No leakage. No peritonitis. Stomach was not distended. Mucosa showed small punctuate hemorrhages. Intestine proximal to section and for 80 cm. distal to it was much congested and showed hemorrhagic areas in it. We are unable to explain these last post mortem findings as being the result of the operation. Our belief, however, is that the early death resulted because of them.

EXPERIMENT 12.—Bitch. Weight 9.2 kg. Fed twenty-six hours before operation. Same operation 36 cm. below pylorus. Postoperative course as above. Lived eighty-two hours. Autopsy. Same findings as in other cases.

EXPERIMENT 13.—Dog. Weight 8.2 kg. Fed twenty-six hours before operation. Bowel sectioned 36 cm. below pylorus and ends inverted. Symptoms same as others. Temperature 35.5° C. Pulse 180 after twenty-four hours. Lived thirty-eight hours. Autopsy. No peritonitis. Ends of bowel tight. At distal closure an intussusception 14 cm. long had taken place and the intestinal wall was much damaged. This, we believe, contributed to an early death.

EXPERIMENT 14.—Young dog. Weight 5.75 kg. Fed seven hours before operation. Bowel sectioned and ends inverted 31 cm. below pylorus. Never fully recovered from operation and died in twelve hours. Autopsy. No peritonitis. Closed ends tight.

EXPERIMENT 15.—Dog. Weight 10.5 kg. Fed forty-eight hours before operation. Bowel sectioned and ends closed 48 cm. below pylorus. Postoperative symptoms same as in other dogs. Lived thirty-two hours. Autopsy. No peritonitis. Closed ends tight. 10 cm. intussusception of distal end in gut, with much damage of gut wall. Proximal intestinal mucosa showed much congestion and many punctate hemorrhages.

These animals were operated on in a practically identical manner at varying periods after eating, and none was fed anything after operation. The one which lived the longest, one hundred and sixty hours, was fed twenty hours before operation, and the one that died in ten hours was fed the last time twenty-seven hours before operation. One dog which ate a full meal only six hours before operation lived one hundred and sixteen hours, while one that had fasted for seventy-two hours lived fifty-five hours after operation. The dogs had all the water they would take, both before and after the operation.

There is seen to be a complete lack of correspondence between the state of digestion at the time the obstruction is produced on the one hand, and the severity of the symptoms and length of life afterward. The same thing was noted in all subsequent experiments. It seems, therefore, reasonably certain that alterations of food residues in the tract above the obstruction has little to do with causing death.

The condition of the dogs after operation can be very briefly summarized. They showed a remarkable absence of active symptoms. Recovery from operation was usually prompt and complete, and on the next day they seemed well, but less lively than before. There was a slight rise in temperature. Water was drunk freely, and promptly vomited, washing

out bile and mucus Emaciation was marked After a varying time the animal began to grow weak, but there was a lack of many of the symptoms we are accustomed to see in the human subject Death seemed to result from general weakness and prostration There was no abdominal distention, and no sign that cramps or colic were present

An autopsy was done in every case, and in none was there any gross alteration in the organs that accounted for death The peritoneum was not inflamed The stomach and the intestine above the section were sometimes but not always markedly distended They usually contained only a very small amount of bile and mucus The mucosa showed a severe congestion In one or two cases there was a superficial ulceration in the intestinal mucosa We were struck with the fact that the most severe changes of this character were present in the dogs living the shortest time We failed, however, to give this observation its full value at that time The gall-bladder was not distended The kidneys and liver showed congestion, and the former evidence of degeneration

From these cases it was determined that animals with a high obstruction might live from five to seven days, that the length of life was not dependent on the presence of food residue in the tract above the obstruction, and that autopsy gave no gross evidence of the cause of death A complete microscopic study of these cases was not made Where sections were examined, however, the same lesions were found in the intestinal mucosa, the kidney and the liver, as those to be described in the second group of cases

We next turned our attention to the production of an obstruction which should not be complicated by any operative damage to the intestinal wall This was considered advisable because there seemed to be a direct relation between the severity of this damage on the one hand, and the severity of the symptoms and the early death of the dogs on the other

A special clamp was devised which proved to be efficient in causing a complete obstruction, without opening the intestine or damaging the blood-supply The clamp consisted of two rubber-covered aluminum plates, about 1 cm in width and 4 cm long Over each end a second covering of rubber tubing was placed One plate was then passed through a slit in the mesentery, with the bowel passing over it The second plate was placed across the bowel at this point, and the two plates bound together near their ends The interval left between them because of the rubber coverings over the ends allowed sufficient room for the intestine, tightly compressed, but not strangulated, to pass through By bending the plates in or out the degree of obstruction was accurately regulated We found by experience that the proper degree was obtained when the thin handle of a scalpel could just be passed within the bite of the plates, but not through it

EXPERIMENTS WITH CLAMP OBSTRUCTION

EXPERIMENT 19.—Male. Weight 13.5 kg. He had been in laboratory seventy-two hours without feeding, and had been given only sterile water to drink. This was done with the idea of getting the stomach and upper intestine as nearly free from bacteria as possible. Cushing and Livingood[15] have shown that the number present can be reduced very markedly in this way. The clamp was placed 18 cm. below the pylorus. The dog remained in excellent condition for five days following the operation. The temperature ranged from 37.5 to 38.5 C. and the pulse from 132 to 150. He drank freely of sterile water, which he vomited soon afterward, washing out bile and mucus with it. He passed small quantities of urine, which contained traces of bile and albumin. During this period he lost 1.8 kg. He showed no active symptoms of the disease and was in no apparent pain. The abdomen was not tender to palpation, nor was it distended. At operation the circumference of the abdomen was 47 cm. and on the fifth day only 43 cm. After the fifth day he began to show marked weakness and a more rapid loss of weight. The pulse and temperature remained about the same, and no new active symptoms developed. On the seventh day he was markedly weak and walked with difficulty. Emaciation was marked, there having been a loss equal to 10 per cent. of his body weight. He was killed with chloroform one hundred and seventy-two hours after the operation and the autopsy, with bacterial examination, immediately done.

The obstruction was tested under water-pressure and was found complete, no water passing through the clamped point. Coils of intestine and omentum were matted around the clamp. When these were separated a small local abscess of green odorless pus was opend. The peritoneal coat of the intestine, at this point, was eroded by pressure, but no serious damage was done to the rest of the wall. The general peritoneal cavity was entirely free from evidence of inflammation. The liver and kidneys showed a marked congestion. There was a marked dilatation of the stomach and upper intestinal segment, and both contained water with bile and mucus. Their mucosæ were markedly congested.

Microscopical Examination.—The intestine proximal to the clamp shows intense congestion and considerable exudative inflammation of the subserous tissue. The submucosa shows hemorrhagic and leukocytic infiltration. There is an acute hyperplasia of the lymph follicles. Distal to the clamp the intestine is normal. The kidneys show intense capillary congestion, especially of the glomeruli. The epithelium is not well preserved.

The spleen shows congestion.

The pancreas is normal.

The liver sections were unsatisfactory.

The cultures from the abscess around the clamp showed a pure culture of a Gram negative bacillus, having the characteristics of colon bacillus. Cultures taken from the inside of the intestine, just above the clamp, showed the same bacterial growth, and in addition, a Gram positive coccus. Our effort to sterilize the upper bowel was not successful, and the local abscess probably resulted from the passage of organisms outward through the intestinal wall, where the peritoneal coat was damaged by the clamp pressure. Other organs were sterile (see table).

EXPERIMENT 20.—Dog. Weight 9.5 kg. No food for one hundred hours. Sterile water during this time. Repetition of Experiment 19. Postoperative symptoms the same. Lived ninety hours. Autopsy (five hours post mortem, during which time the body was on ice) showed no peritonitis. Obstruction complete. Stomach a little dilated. Bowel above clamp twice the normal size. In the intestine, distal to the clamp, were seen many hemorrhagic points.

15 Cushing and Livingood. Johns Hopkins Hosp. Rep., 1900, ix, 543.

Microscopic Examination.—Intestine proximal to clamp shows fibrinous peritonitis, marked congestion of all coats, and exfoliation of lining mucosal cells Stomach normal

Liver Congestion Moderate granular and fatty degeneration

Kidney Moderate congestion Tubules show granular coagulum Cells swollen and show marked granular degeneration Some cells show partial necrosis

Cultures from femoral blood, peritoneum and liver show neither aerobic nor anaerobic growth

EXPERIMENT 21.—Puppy Weight 8 kg Fed four hours before operation Clamp applied 16 cm below pylorus The stomach contained food and the lymphatics and vessels were distended from full digestion The postoperative course was the same as in the others Pulse, 190 Temperature, 39 C He died fifty eight hours after operation Autopsy, eight hours post mortem, the body being on ice No peritonitis Moderate distention stomach and duodenum Few drops of purulent-looking fluid around clamp, buried in adhesions The obstruction was complete The gastric and intestinal mucosa, proximal to the clamp, showed very little change Distal to the clamp there were areas which resembled submucous hemorrhages in the intestinal wall The other abdominal organs appeared normal

Microscopic Examination.—Proximal intestine not examined Distal intestinal mucosa shows hyperplasia of lymph follicle, but no hemorrhages

Liver Marked congestion

Kidney Congested Tubule cells swollen, and in places intensely degenerated Apparent necrosis present in some foci of the tubules

Spleen and pancreas much congested

Bacteriology.—Cultures from femoral blood, peritoneum and liver all sterile, both aerobic and anaerobic, with exception of one colony on one peritoneal aerobic slant agar tube This showed pure growth of a Gram-positive coccus and probably was an air contamination, one of the three found in the whole series

EXPERIMENT 22.—Bitch Weight 10 25 kg Fed eighteen hours before operation Clamp applied 12 cm below pylorus This animal was killed on the twenty-first day, up to which time she had showed no symptoms of suffering or of disease, except for loss of weight to 7 4 kg During this time she had eaten nothing She drank water freely, however, for the first three days After this she received 150 to 250 c c of normal saline solution subcutaneously, and then she refused water to drink She only occasionally vomited The temperature ranged between 37 and 38 C The pulse for the first four days was about 130 It then became slower and during the last two weeks was between 70 and 100 Urine was passed freely and contained bile and albumin in small quantities Several blood cell counts were made during the later days of the experiment and the red cells numbered 5,240,000 and 7,000,000, nearly all counts being slightly over 5,000,000 Killed with ether on the twenty-first day while still in good health Autopsy No peritonitis Clamp allowed minimal drainage into intestine below (compare Experiment 27) Stomach and duodenum were somewhat dilated and the intestine distal to clamp was contracted, showing very little flow into it It contained bile, however, and under pressure water was forced through the clamp into the bowel below The mucosa of intestine and stomach appeared normal The kidneys and liver showed congestion

Microscopic Examination.—Stomach and intestine not examined

Kidneys Tubules filled with granular coagulum Cells show swelling and granular degeneration

Liver Intensely congested

Spleen Congested

Pancreas Normal

Bacteriology.—Neither aerobic nor anaerobic growth on tubes from femoral blood, liver or spleen

EXPERIMENT 23.—Dog. Weight 11 kg. Clamp applied 20 cm. below pylorus. He was killed on the tenth day. His symptoms were the same as in cases above. He received saline subcutaneously with apparent benefit. Pulse, 132 to 150. Temperature 37.9 to 39.2 C. Weight at death 8.5 kg. Urine contained albumin and bile. An extensive noma developed on face which accounted for high temperature. Autopsy immediately after death by cyanid. Obstruction complete. Stomach and proximal intestine much dilated. They contained about 300 c.c. of bile, mucus and water. Distal intestine collapsed. The specimen was preserved intact so the mucosa was not seen. No peritonitis. Liver and kidneys much congested. The latter showed acute degeneration, the markings being indistinct and the papillae pale.

Microscopic Examination.—Kidneys showed granular degeneration, in places very marked.

Liver. Intensely congested. Considerable fatty and granular degeneration.

Spleen and pancreas show congestion.

Bacteriology.—Aerobic and anaerobic cultures from femoral blood and liver were all without growth.

EXPERIMENT 24.—Small bitch. Weight 6 kg. Fed seventy-two hours before operation. Clamp applied 16 cm. below pylorus. Lived 205 hours—a little less than nine days—with symptoms as in other cases. Pulse, 120 to 180. Temperature average, 39 C. Received saline injections throughout. Red blood-cells increased from 5,000,000 before operation to 6,720,000 on eighth day, showing some loss of water. Weight 4.9 kg. Died during night. Autopsy at 10 in morning. Obstruction complete as tested by absence of bile in distal intestine, although under pressure water could be forced beyond the clamp. No peritonitis. Around the clamp in a mass of omentum there was a collection of dirty yellowish fluid. The intestinal wall showed some damage within the bite of the clamp (see microscopic examination). The stomach and proximal intestine were moderately dilated, and contained a small amount of bile and mucus. The mucous membrane of the whole tract appeared normal to gross examination, as did the kidneys and liver.

Microscopic examination.—The intestine within the clamp showed an advanced purulent peritonitis, destroying longitudinal muscular coat. Congestion of mucosa. Exfoliation of epithelium.

Kidney. Congested. Tubules filled with granular coagulum. Cells swollen and show marked granular degeneration, in places approaching necrosis.

Liver. Section lost.

Pancreas. Marked congestion.

Bacteriology.—Aerobic and anaerobic cultures from femoral blood, peritoneum and liver showed no growth. A Gram-positive coccus was obtained in pure culture from the abscess around the clamp in all tubes both aerobic and anaerobic. This was undoubtedly an infection passing through the intestinal wall where the clamp damaged it, since the same organism was isolated from the intestine.

EXPERIMENT 25.—Dog. Weight 6 kg. Fed twenty-seven hours before operation. Clamp applied 16 cm. below the pylorus. He took ether badly and made a poor recovery. He was not given the hypodermoclysis of normal saline solution. Condition seemed good the next day. Died that night. Autopsy not done till day following, about thirty hours post mortem. Obstruction complete under water-pressure. No peritonitis. Stomach and proximal intestine somewhat distended. Distal gut collapsed. Mucosa of stomach and proximal gut markedly congested. Liver, spleen, pancreas and kidneys show congestion.

Microscopic Examination.—Stomach congested. Proximal intestinal mucosa shows intense congestion. Marked round-cell infiltration of tips of villi and exfoliation of cells.

Liver. Intense congestion. Considerable fatty and granular degeneration.

Kidney. Not examined.

No bacteriological examinations made because of delayed autopsy.

EXPERIMENT 26.—Bitch. Weight 7.2 kg. Exact duplicate in every way of Experiment 25, except that she received the saline injections daily, the first one of 150 c.c. being given at the close of the operation. She lived ten days, and showed the same symptoms as did other dogs described. Killed with ether and autopsied immediately. Obstruction complete against a column of water 90 cm. high. No peritonitis. The stomach and proximal intestine were dilated to about three times normal. The mucosa of stomach and proximal intestine were congested. The liver, kidneys and spleen were congested.

Microscopic Examination.—Proximal intestinal mucosa shows intense congestion and exfoliation of living cells.

Kidney. Shows congestion. Tubules contain coagulum and occasional blood-cells and show granular degeneration, in places very marked.

Liver shows intense congestion, atrophy of liver cords about distended capillaries and moderate granular degeneration.

Spleen shows much hypertrophy of the follicles.

Pancreas normal.

Cultures from femoral blood, spleen, liver and peritoneum were taken. These were all without growth after four days, under both aerobic and anaerobic conditions, except one aerobic tube (four were taken) from the liver. This showed one colony of Gram positive cocci growing well to one side of the stroke of the loop.

EXPERIMENT 27.—Bitch. Weight 11.5 kg. Fed twenty-eight hours before operation. Clamp applied about 15 cm. below the pylorus. Lived twenty-four days with only moderate development of symptoms during first week. Then behaved like a normal dog. Reoperated on July 20. The clamp was not sufficiently tight to make a complete obstruction (compare with Experiment 22). It was tightened and the next day it had cut its way through the intestinal wall and the dog died of a peritonitis.

EXPERIMENT 28.—Dog. Weight 6.5 kg. Fed twenty-eight hours before operation. Clamp applied 11 cm. below pylorus. Postoperative course as in other cases. Died in fifty-six hours and body immediately placed on ice. Autopsy ten hours later. Obstruction complete. No peritonitis. Local infection around the clamp. Stomach and proximal intestine only slightly distended and contained a small amount of bile stained fluid. The distal intestine was totally empty. Two superficial ulcers were seen in the mucosa of proximal intestine. The liver, kidneys, spleen and pancreas appeared normal.

Microscopic Examination.—Intestine not examined.

Kidney. Tubules swollen and show granular degeneration, very marked in places.

Liver. Intensely congested.

Bacteriology.—Cultures from the femoral blood, the peritoneum, the liver, the spleen and the local collection of fluid around the clamp all were without growth under aerobic and anaerobic conditions.

EXPERIMENT 29.—Dog. Weight 8 kg. Fed thirty hours before operation. Clamp applied 11.5 cm. below pylorus. Postoperative course same as in other cases. Received about one fortieth of body weight of normal saline subcutaneously daily. He remained in excellent condition for eight days, during which time he lost a little over 2 kg. in weight. He drank water each day, which was always vomited. Pulse from 130 to 174. Temperature, 38.5 to 40 C. On the ninth day he was etherized and the abdomen opened. The obstruction was complete. The stomach and proximal intestine were much distended and filled with a greenish fluid of the thickness of pea soup. There was no peritonitis. The splanchnic vessels were not much dilated, except those above the occlusion. The spleen was not enlarged. The liver and kidneys did not show much congestion. Sections were taken from the stomach, duodenum, distal intestine, liver, spleen and kidney while the dog was still under ether, after which he was killed.

Microscopic Examination.—The stomach is normal.

The proximal intestine shows marked congestion and an edema in the submucosa The lesions here were not advanced, because the specimens were taken some hours before the animal would have died from the disease The distal intestine is normal

The liver is congested The cells show granular degeneration and areas of necrosis are present

The kidney is congested Tubules contain granular coagula The cells show granular degeneration

The preceding series includes experiments on eleven animals which may be summarized In two a complete obstruction was not obtained because the clamp was not sufficiently tight The two animals were killed after twenty-one and twenty-four days, respectively, while each was in good condition They had lost considerable weight, and one of them was weak, but otherwise they acted like normal dogs

The remaining nine dogs all showed a similar condition while living, and autopsy yielded the same findings in the organs In some dogs, particularly those which lived the longest, the dilatation of the stomach and the duodenum was very marked

Four dogs in this series were given sterile water by mouth only, and this they promptly vomited They were, therefore, practically getting neither nourishment nor water, for if what they drank was not all vomited, very little absorption could take place from the stomach and duodenum Beginning with the fourth dog, each dog received from 150 to 300 c c sterile normal saline solution, subcutaneously, per day This was absorbed In one case this was not given till the third day This animal had then begun to act sick, but showed a marked improvement after the administration of the hypodermoclysis In general, the dogs which received this saline lived longer than the others They sometimes drank sterile water in addition, but this they always vomited in a very short time In many cases they refused to drink more than a few cubic centimeters The three dogs which lived the longest were all given the saline subcutaneously, and there seems no doubt that this procedure was very beneficial

We attempted, by a count of the red blood-cells, to determine whether the dogs not receiving it suffered from dehydration, with a consequent increase in the number of red cells per cubic centimeter There was not a sufficient variation from the normal to throw any light on this point, the number ranging around 6,000,000 both before and after the saline injections The longest postoperative life was ten days (two dogs)' a third dog lived just under nine days, the shortest period was thirty-six hours in a small fox terrier which never completely recovered from the operation The average length of life was one hundred and thirty-nine hours in this series, as against sixty-seven hours in the series in which the obstruction was produced by sectioning the intestine and inverting the ends It was only in this series that the saline injections were used

It was, moreover, remarked that the second series, in which no damage to the intestinal wall was produced, was much nearer the normal throughout the experiment than was the first series, in which the intestine was sectioned So far as we know, no other investigator has paid the same attention to producing an obstruction without any damage to the intestinal wall There is evidence that the method is of advantage in excluding the complications which may arise from such damage Clinically, we know that when a strangulation, however limited in extent, is superimposed on an obstruction, the course of the disease becomes much more acute Medowoy,[16] Kocher[17] and others emphasize this point

Murphy and Vincent,[12] in their experiments, worked particularly on this phase of the question In a simple obstruction in cats they found the intestinal wall very little damaged in four to six hours When, however, the circulation was disturbed, particularly a venous stasis produced a very severe change was present at this time They conclude that "interference with the circulation of the obstructed intestine is the vital factor in the production of the symptoms of ileus"

When the intestine is sectioned, and the proximal end sufficiently inverted to prevent the stomach and duodenal contents being forced through it, a local strangulation takes place At autopsy there was always found in these cases a local damage to the intestinal wall at the inverted point, small in extent, to be sure, but nevertheless sufficient to be the possible site of the absorption of a considerable amount of poison Often, too, a very considerable intussusception of the invaginated stump was present This mechanical damage, of course, is not an essential in producing death, because it was not present in the second series, in which the animals died We feel, however, that it has an important bearing on the problem, as we shall later more fully explain

We desire to call particular attention to the microscopic findings in these cases The intestinal mucosa, proximal to the obstruction, shows a marked congestion Marked round-celled infiltration is present, and exfoliation of the lining cells Hemorrhages are often seen There is present an acute hyperplasia of the lymph follicles The submucosa is edematous Distal to the clamp there is no change, or only a very slight change, from the normal The gastric mucosa shows only a congestion The kidneys are markedly congested The tubules contain granular coagula The tubule cells are swollen and the seat of granular degeneration, not infrequently necrotic areas are seen

The liver is congested and the cells show granular and fatty degeneration Necrotic areas may be present

16 Medowoy Deutsch Ztschr f Chir, 1910, cv, 1
17 Kocher Mitt a d Grenzgeb d Med u Chir, 1898 99, iv, No 2, p 195

The spleen shows marked congestion, and some hyperplasia of the lymph follicles

The pancreas shows only congestion

The changes in the intestinal mucosa are worthy of careful study They may result from two causative factors The marked distention and stretching of the intestinal wall may produce a circulatory disturbance or possibly an actual trauma to the mucosa The stagnated secretions may be sufficiently irritating to damage the mucosal cells chemically In either case there would develop a condition of lessened resistance, and the intestinal bacteria would be free to attack the mucosa and bring about the changes seen This having occurred, the changes in the other organs may be conceived as having been caused by the entrance into the blood of poisonous materials which were produced by this bacterial activity Sufficient evidence that such an explanation is correct is found in the fact, already pointed out, that there is an apparent parallel between the extent of the changes in the mucosa and the severity of the symptoms

The evil effects of hyperdistention to the intestinal circulation has been experimentally shown by Van Zwalenburg [18] By means of illumination in the intestinal lumen he watched the circulation when different degrees of pressure were exerted inside the bowel At a pressure of 30 mm mercury it was noted that some capillary streams were arrested, while at 60 mm small veins became blocked Above this the circulation showed increasing disturbance If a continuous pressure of from 80 to 90 mm were continued for an hour, the apparent number of veins seen on the serous surface was greatly increased Their caliber was also greater than normal This observation would seem to indicate that the effect of the long-continued distention in a blocked bowel must seriously damage the integrity of the mucosal circulation and lead to the lesions observed in our experiments and recorded above

The influence of the stagnated secretions above the obstruction may be considered as important in giving rise to poisonous materials which could enter the circulation through the damaged intestinal wall In order to study this phase of the question, we turned our attention to an elimination of the bile, pancreatic and duodenal secretions as a source of the poisons To this end, in five dogs the following operation was done The pylorus was divided and the two cut ends closed by inversion The intestine was divided just distal to the duodenum, and the proximal end implanted in the jejunum about 30 cm lower down. The distal end was implanted into the stomach By this means the stomach emptied into the intestine just below the duodenum, and the bile, pancreatic and duodenal secretions passed into the jejunum about 30 cm distal to this Our intention was to cause an obstruction with the clamp, just orally

18 Van Zwalenburg Ann Surg, November, 1907, xlvi, 780

to the new duodenal outlet when the animals should have recovered from the anastomoses, thus having an obstruction of the stomach and about 30 cm of the intestine but not of the duct-bearing portion of the tract For a few days following the operation these dogs did well, fully regained their appetites and seemed to be in good health After about a week, however, they began to act sick They did not eat well, and sometimes vomited They lost weight rapidly Some of them developed diarrhea with bloody-appearing stools Four of them died in about four weeks with evidence of malnutrition The autopsy showed, in two of these, that a very extensive damage to the intestine between the stomach and duodenal anastomosis was present Ulcers were seen which were about to perforate The stomach and this part of the intestine contained partially digested blood

The fifth dog seemed to be in fair condition after two weeks, and on the nineteenth day the abdomen was opened under ether anesthesia The anastomoses were satisfactory and no evidence of ulceration was present from the peritoneal surface The occlusion clamp was applied just above the anastomosis of the duodenum with the jejunum The following day the dog was in good condition, he had not vomited He was given saline subcutaneously He died during that night and autopsy was done in the morning The anastomoses were satisfactory A general peritonitis was present from a perforation within the bite of the clamp The stomach and intestine anastomosed to it were distended with a dirty brownish fluid When they were opened the gastric mucosa was seen to be much congested, the mucosa of the jejunal segment was markedly congested and contained many hemorrhagic areas The intestine below the duodenal anastomosis was entirely normal in appearance The effort to produce an obstruction in which the duodenal contents should not be stagnated had not been successful But the symptoms developing in these dogs and the findings in the intestinal mucosa added emphasis to the relations existing between these two factors In many ways these dogs behaved like the ones with an obstruction, the symptoms and the course of the disease being, however, much less acute The microscopic examination of the intestinal mucosa, the kidneys and the liver, showed, too, the same lesions as found in the simple obstruction

The fact that such damage to the intestinal wall, in so far as it occurs in intestinal obstruction, is the cause of serious symptoms, and, if extensive enough, may even cause death, seemed to be well demonstrated That it is the sole factor, however, was not proved, and as stated above, our attention had been arrested by the benefit derived from the administration of salt solution under the skin It was conceivable that this benefit arose from diluting and eliminating the poisons absorbed through the damaged intestine Such a supposition, however, had no support in any work done, and accordingly a more detailed study of this question was

undertaken, and for this purpose Dr Benedict, of the department of chemistry, kindly offered to make some studies in metabolism for us To him we are indebted for a most important discovery which, we believe, throws much light on the question under consideration, if it does not completely solve the problem of the cause of death from uncomplicated intestinal obstruction

EXPERIMENT 1.—The first dog under his observation was a female in good condition, weighing 12 1 kg The clamp was applied under ether narcosis on the lower part of the duodenum, March 4, 1912 Table 3 gives the results found during the three and a half days that she lived

TABLE 3.—RESULTS OBTAINED AFTER CONSTRICTION OF THE LOWER PART OF THE DUODENUM FOR THREE AND ONE HALF DAYS

Date	Weight, kg	Urine, c c	Vomitus, c c	Combined Fluid Output, c c	Total Nitrogen, gm	Remarks
3/4/12	12 10					Clamp applied to lower duodenum
3/5/12	11 28	26	720	746		Received 100 c c of a 0 9 per cent saline solution
3/6/12	10 36	15	500	315	10 334	Received 100 c c of a 0 9 per cent saline solution
3/7/12	9 86	86	510	596		Received 100 c c of a 0 9 per cent saline solution
3/8/12						Died
Totals,		127	1,730	1,857		

From these figures it is seen that she lost 2 2 kg in weight Her output of fluids, including the urine and vomitus but not counting the water of expiration, was 1,857 c c, to offset which she had only received 300 c c subcutaneously The total nitrogen output in two days was 10 31 gm, which equals, approximately, 0 5 gm per kilo per day, which is two or three times the normal output A sample volume of urine of 127 c c contained 3 574 gm albumin The creatin excreted in this equaled 0 638 gm, which was more than double the amount of creatinin

It was obvious from these figures that a very severe grade of tissue disintegration was taking place, and that a water loss averaging about 5 per cent of the body weight was suffered each day It at once became apparent that the amounts of water we had been giving were entirely inadequate to replace this great loss, and that probably death was much hastened by such a loss

Accordingly, we made a study of three dogs in which the daily output was measured, and a quantity of normal saline about 100 c c in excess of this was injected under the skin These dogs with the clamp applied on the lower duodenum were kept alive in this way for three weeks (one for twenty-six days), and during this time they remained in good condition They were then etherized a second time, and the abdomen opened to see if the obstruction were complete In every case it was found to be so The stomach and the duodenum above the clamp were excessively dilated, and their contents could not be forced through the

clamp into the intestine below, which was found empty and much contracted By varying the amount of water injected, we could accurately control the condition of the dogs The complete study of one of the experiments is shown below

TABLE 4.—RESULTS OBTAINED WITH CLAMP ON THE DUODENUM FOR THREE WEEKS AND INJECTIONS OF SODIUM CHLORID

Date	Weight, kg	Urine, c c	Vomitus, c c	Combined Fluid Output, c c	Injection 0 9% NaCl Sol , c c	Remarks
3/13/12	11 3					Clamp applied to lower duodenum
3/14/12		120	420	540	250	
3/15/12		175	—	175	200	
3/16/12		160	600	760	200	
3/17/12	10 46	170	—	170	200	
3/18/12	10 84	135	—	135	500	
3/19/12		†	†		150	
3/20/12		130	850*	980	250	
3/21/12	9 64	130	—	130	550	
3/22/12	10 24	100	550*	650	850	
3/23/12		80	800*	880	500	
3/24/12	10 24				600	
3/25/12	9 68	70	1,180*	1,250	750	
3/26/12		†	†		800	
3/27/12		85	1,160*	1,245	800	
3/28/12	10 00	310	—	310	700	
3/29/12		562	150*	712	300	
3/30/12		390	—*	390	200	
3/31/12		150	200*	350	200	
4/ 1 /12		55	80*	135	150	
4/ 2 /12	8 9	55	—*	55	100	Is in excellent condition
4/ 3 /12	8 3		650	650	—	Obstruction found complete Clamp removed Recovery
Total for 21 days		2 877	6,640	9,517	8,250	

* Obtained by stomach tube † Incomplete — None

EXPERIMENT 2.—A bitch weighing 11 3 kg had the clamp applied under ether narcosis, on the lower duodenum, March 13 April 3, twenty one days later, when in excellent condition, she was again etherized and the abdomen reopened The obstruction was found to be complete The clamp was removed and she is still alive The total vomitus during the twenty one days was 6,640 c c and she received to offset this 8,250 c c of saline solution Much of the vomitus had to be obtained by the stomach tube, as the stomach became dilated and would not empty itself She lost 3 kg in weight The study of the urine gave most interesting results During the first week it showed very marked abnormalities The albumin content was as high as 4 5 gm per day, and the total nitrogen, exclusive of this, was, on an average, 5 gm The creatinin creatin ratio was about 1 to 2 instead of 10 to 1, which is near the normal for a starving dog The latter ratio was later established During this week the vomitus was 1,870 c c in amount, which was 120 c c more than the amount of saline solution administered The urine equaled 890 c c, so that there was a loss of water equal to one tenth of the body weight The condition of the animal was only fair during this week After the seventh day the saline was increased to 550 c c daily and maintained there, or higher, for several days The abnormalities in the urine entirely disappeared, and it became the urine of a dog under normal starvation, except that it contained bile throughout There was coincident improvement in her condition Later, on the sixteenth day when vomiting had ceased and the stomach-tube recovered very small amounts, the saline was again decreased to 200 c c or less, the dog remained well, and the urine continued normal

EXPERIMENT 3.—A small bitch weighing 5 88 kg had the clamp applied under ether narcosis to the lower duodenum, April 8, 1912 She received the normal

saline solution subcutaneously, every day, the first injection of 300 c c being given immediately after the operation The quantity was repeated on the two following days, and then for sixteen days she received 400 c c daily, and for two additional days 300 c c each The total saline administered during the continuance of the experiment for twenty-one days was 7,900 c c an average of 376 c c per day, which was equal to about 8 per cent of her average body-weight. During this time the total amount of fluids excreted in the urine and the vomitus was 6,354 c c Much of the vomitus had to be excreted by means of the stomach-tube because the stomach became atonic and vomiting ceased even though the organ was full Her loss in weight was 1 4 kg Throughout the entire experiment she remained in a condition of apparent perfect health She was always playful and lively On the twenty-first day she was etherized and the abdomen was reopened The clamp was found *in situ* and the obstruction was complete Four hundred and fifty c c of water were put in the stomach through the tube This readily passed through the pylorus and down the duodenum to the clamp, where it was completely stopped It was impossible to force any beyond the clamp by direct pressure on the duodenal wall The clamp was removed and a small perforation, which was completely closed by the rubber covering of the plate and by adhesions, was found in the bite of the clamp There was no peritonitis

EXPERIMENT 4.—A bitch weighing 12 2 kg had the clamp applied under ether narcosis to the lower duodenum, April 19, 1912 During the following six days she received 1,500 c c of normal saline solution subcutaneously, and excreted in the form of vomitus and urine 1,020 c c On the seventh day she did not vomit, and the stomach-tube recovered nothing We suspected that the clamp was not tight enough to produce a complete obstruction, and she was accordingly given 500 c c of water through the stomach-tube and a quantity of meat which she ate greedily Following this she again vomited, and in four days put out 2,150 c c in which the digested meat was present The injections were then increased, quantities varying from 700 to 500 c c being given daily The vomiting ceased and a quantity of urine was passed, slightly less than the saline injected, the total for seven days being, saline 4,000 c c, urine 3,200 c c The saline was again decreased, and the urine fell, but no vomiting recurred She was etherized May 16, twenty-six days after the obstruction, and the abdomen was reopened The obstruction was found to be complete The stomach and duodenum were found to be enormously dilated, having a capacity of 1,000 c c, while the intestine below the clamp was contracted and empty, not showing any trace of bile No detailed study of the urine was made in this or the preceding case

It will be noted that in many instances there was no vomiting, but the stomach-tube recovered large quantities This must be taken into account in determining the amount of saline to be administered, because whether vomited or retained in the stomach, it means a loss to the animal These three dogs were under identical conditions with the nine previously reported, except that they received amounts of normal saline solution about 100 c c in excess of the daily output of vomitus, often reaching 500 to 700 c c per day, while the animals in the former series received only 150 or 200 c c per day No dog in the first series lived longer than ten days All dogs in the later number were alive and in good condition at the end of three weeks, and apparently would have succumbed only to starvation if the experiment had been continued

In order to be certain that no leakage took place, an obstruction was produced in two dogs by dividing the lower duodenum and closing the

ends by inversion The result was the same as in the dogs having the clamp applied Care must be taken, however, to minimize the amount of damage done to the intestinal wall in this operation, otherwise the animal is poisoned from this source and may die

From these results it is plain that the loss of fluid from the organism is the disturbing influence and is the important factor in explaining the symptoms and death following uncomplicated high intestinal obstruction in dogs Death is caused by the loss of water which the tissues sustain from the excessive amounts vomited, and death can be prevented if this water be replaced by cutaneous injections of normal salt solution

The source of such enormous amounts of vomitus is a matter of interest Dr Benedict's study of the vomitus from the experiment detailed under Table 4 showed it to be strongly acid, and to have a specific gravity of 1 007 The total amount contained 16 72 gm of chlorin, which, computed as sodium chlorid, equals 27 65 gm Bile was always present The low specific gravity indicates that it was largely water These findings show, as would be expected, that the vomitus is a diluted mixture of bile and gastric secretion The part played by the pancreas and duodenum is unknown In all the cases the vomitus was more abundant during the early part of the experiment than it was later, and toward the end it often almost entirely disappeared The following considerations offer an explanation of this unexpected fact The dogs were all in good condition when operated on, and presumably the liver and other glands were actively secreting The products of these glands, bile, pancreatic juice and duodenal secretion, found no outlet beyond the duodenum, and hence were forced back into the stomach Here they acted as an irritant to the gastric mucosa, and as a result a secretion of gastric juice and an excretion of water took place to the enormous amounts found As the experiment proceeded the dog gradually developed the condition of a starving animal in which all the alimentary glands become inactive, the bile in particular being very small in amount The disturbing influence of these retained secretions being absent, the vomiting gradually grows less, and finally almost ceases The small amount of bile secreted is taken care of in the duodenum and stomach, being either digested or absorbed That some is absorbed is shown by the fact that the urine contains bile throughout the experiment The bile alone, however, is not the offending factor, because in cases in which the common bile-duct was tied and an anastomosis made between the gall-bladder and the intestine below the clamp, large amounts of vomitus appeared, though not equaling those above given

The results of all our work demonstrated, therefore, that there are two important factors concerned in producing the symptoms and causing death in a high intestinal obstruction in dogs

First, the loss of water from the tissues caused by the excessive drainage into the intestinal lumen followed by vomiting This factor is entirely under therapeutic control It can be rendered harmless by the subcutaneous administration of normal saline solution

Second, the absorption of poisonous materials from the intestinal wall, when, by reason of overdistention and chemical irritation the mucosa is damaged and bacteria set up an active inflammation in it, and possibly the other intestinal coats This factor is not appreciably influenced by the administration of the salt solution

REVIEW OF THE WORK OF WHIPPLE, STONE AND BERNHEIM

We have referred above to the work done by Whipple, Stone and Bernheim, which appeared[3] after preliminary reports of our work had been published,[19, 20] setting forth the conclusions just given As their conclusions differ in important particulars from ours, it seems advisable to compare the two, and if possible reconcile the facts as a basis for the correct conclusion In doing this we shall quote freely from an article already published,[21] in which this phase of the subject was treated

Whipple, Stone and Bernheim,[22] studied the question by producing a closed loop of the duodenum, beginning just below the pancreatic ducts and ending beyond the suspensory ligament This portion was isolated from the remainder of the intestinal tract by tying a heavy ligature tightly at these two points, and burying it under an inverted layer of the peritoneum A gastro-enterostomy was then done Dogs so treated died in from thirty-six to seventy-two hours The authors inferred from this that there developed in the closed loop a poisonous material, the absorption of which caused death Since the closed loop contained neither gastric secretion, bile nor pancreatic juice, the conclusion seemed inevitable that some alteration in the loop function was responsible for death

A series of careful and well-controlled experiments was then undertaken to study the nature, and if possible, to determine the source of any poison The method employed was to produce the isolated loop, collect the contents, sterilize them by heating to 60 C, filtering them, and then, after autolysis, to inject them into a healthy dog, intravenously, subcutaneously or peritoneally It was demonstrated in this way that dogs so injected died promptly Further, the authors were unable to isolate any poisonous material from the normal intestine by any method of treatment, and if the mucosa of the loop were first destroyed by a

19 Hartwell and Hoguet Am Jour Med Sc, 1912, cxliii, 357

20 Hartwell and Hoguet Experimental Intestinal Obstruction in Dogs, with Especial Reference to the Cause of Death and the Treatment by Large Amounts of Normal Saline Solution, Jour Am Med Assn, 1912, lix, 82

21. Hantwell, John A Jour Exper Med, 1913, xviii, No 2, p 139

22 Whipple, Stone and Bernheim Jour Exper Med, 1913, xvii, 286

fluorid, no poisons appeared Lastly, some dogs died within a few days when the loop was drained externally, there being, according to the authors, an absorption directly from the mucosa, and not from the lumen

The authors summarize, in part, as follows

There seems, therefore, no escape from the conclusion that a poisonous substance is formed in this closed duodenal loop which is absorbed from it and causes intoxication and death Injection of this toxic substance into a normal dog gives intoxication and a reaction more intense than, but similar to, that developing in a closed loop dog (p 306)

The mucosa of closed or drained duodenal loops contains a toxic substance quite similar to the toxic material found in the lumen of the closed loops

Normal intestinal mucosa contains no toxic substance nor can it neutralize *in vitro* the toxic substance produced in the closed loops Our experiments show that formation and absorption of a toxic substance may proceed in a loop whose blood supply has in no way been disturbed, and whose mucosa is quite intact both in gross and microscopically (p 323)

The authors apply their findings to the condition of simple intestinal obstruction, and state it as their belief that "the toxic material concerned is the same in both instances" (p 321) They add "Our experiments indicate that intoxication is the primary effect, and the loss of body fluids a secondary one, rather than the reverse, as is claimed by Hartwell and Hoguet"[20] In substance, they claim to have proved that a closed loop of intestine, without undergoing any morphological change, produces a toxic substance which is absorbed and causes death Moreover, they infer that a similar condition exists in simple intestinal obstruction, and that here also death results from the absorption of this same poison

We fail to find any evidence in their experiments, as published, to justify these claims On the contrary, we find that the protocols definitely show a damage to the intestinal wall, and that the toxic substance they are dealing with arises from this source In the absence of this damage no toxemia is present except that produced by the loss of water in the vomitus We submit the following review of their protocols in support of this view

Dog S 34.—Moribund in twenty seven hours Killed with ether Autopsy immediately "The duodenal loop is of enormous size" "Purplish patches show through the transparent serosa" "The contents are blood stained"

Microscopical Examination.—"In places the wall of the duodenum shows necrosis, hemorrhage and invasion by leukocytes"

Dog S 27.—Died in thirty-two hours

Autopsy.—"The duodenal loop is contracted and contains a little material Its walls show a few areas of subserous hemorrhage, about 1 or 2 cm in diameter There is obvious necrosis associated with these areas, and through one of them rupture had taken place, with escape of duodenal loop fluid into the peritoneum" No microscopical examination is given

Dog S 20.—Death in forty-eight hours One hundred c c of fluid are in the loop "The closed loop mucosa is pinkish and a little swollen, but no ulcers are present" The proximal loop was not tight, and some fluid had probably regurgitated into the stomach

Dog 56—Death in fifty-five hours Remained well for twenty-four hours

Autopsy—"The abdominal cavity is clear except for slight granular peritonitis about the loop and area of operation" "The small intestine below the loop contains blood-stained mucus, and its mucosa has a mottled appearance, with large areas of deep red color where the capillary injection is quite marked The duodenal loop is rather flabby and not greatly distended It contains about 50 c c of a thin, soup-like, faintly blood-tinged fluid The loop mucosa is quite intact, but of a delicate pinkish tinge" No microscopical examination is given

Dog S-53—Death in three days Seemed well for twenty-four hours

Autopsy—The condition of the loop mucosa is not given, it is merely stated that it is pink and intact No microscopical examination is given

Commenting on these four experiments the authors say

The duodenal loop may show a normal intact mucosa in gross and microscopically, except for a little diffuse injection Again, it may show necrosis, ulceration and hemorrhage, and the contained fluid may vary correspondingly in characteristics, but it always contains a toxic substance (p 291)

In only one case, however, is a microscopical examination given, and then marked changes were found, while in the others evidence of change was seen grossly The length of life seems directly to correspond to the lack of damage found in the mucosa, as demonstrated by the reported findings in Dog S-53, which lived for three days

The following protocols give the conditions found in the duodenal loop, when the dog is killed at the end of twenty-four to twenty-eight hours In one case, Dog S-4, the contents were blood-tinged, owing to inversion of the duodenal mucosa

Dog S 4—"The mucosa throughout the loop is intact, but slightly swollen and of a pinkish color"

Microscopical Examination—"The tips of a few of the villi are slightly swollen and contain a few polymorphonuclear leukocytes The epithelium is normal throughout"

Dog S 2—In twenty six hours the duodenal loop contained blood-tinged fluid, in places, on microscopical examination, "it shows some areas of necrosis with hemorrhage and invasion by pus cells, involving mucosa and submucosa"

Dog S 5—The loop contained blood-tinged fluid

Microscopical Examination—"The duodenal loop is quite normal"

In two dogs, S-28 and S-29, the duodenal loop contents were not blood-tinged, and the mucosa, on gross examination, seemed normal, though no microscopical examination is given

These dogs were all in fair condition when killed, being described as "dull and drowsy," "weak," "pulse strong," "somewhat dull but not very toxic" The incipient damage to the mucosa, as shown in these cases by the blood-tinged fluid and the microscopical examination, explains the cause of their condition We cannot reconcile a normal mucosa with a blood-tinged content, as reported in Dog S-5

Dogs S-12 and S 9—Both dogs were killed after forty-four hours, and were apparently in fair condition, Dog S-9 showing a carotid pressure of 100 to 110 mm of mercury, with almost normal pulsation There were no visible changes in the loop mucosa, either on gross examination or microscopically

Dog S 38.—The dog was killed after forty eight hours, his condition being "somewhat toxic" "The blood pressure was practically normal and the pulse regular" "The duodenal loop contains 20 to 30 c c of a strawberry colored thick fluid The loop shows a diffuse pinkish mucosa, no ecchymoses and no ulcers"

These dogs were killed after from forty-four to forty-eight hours, when in good condition, and no change was found in the mucosa If the enclosed secretion from the mucosa itself is the active poison, the absorption of which, by the mucosa, causes death, why are the dogs not made more ill? We believe it is because in these individual cases the mucosa was not so severely damaged If we compare them with Group 1, in which the dogs were allowed to die, and with the group immediately preceding, in which at the end of approximately twenty-four hours the dogs were apparently more toxic, and a definite change in the mucosa was found, we cannot escape the conclusion, from a careful analysis of the cases, that the severity of the toxic symptoms is in direct ratio to the damage done to the mucosa

In their injection experiments (pp 301-304) the authors prove beyond question that they have obtained from the contents of the closed loop a substance, which, after sterilization by heat (60 C for thirty minutes) and filtering through a Gooch or other porcelain filter, is very poisonous, and will cause death in a few hours, if it be injected into the vein, the peritoneum or the subcutis of a dog

Whipple, Stone and Bernheim find that a similar poison can be extracted from the mucosa (not the loop content) of a non-drained or a drained closed loop, injection of which into dogs produces severe toxic symptoms (pp 310-311) They argue from this that the substance with which they are dealing is elaborated in the mucosa of the closed loop, and that some unknown force is at work which profoundly alters the activity of the epithelial cells Yet they emphasize the fact that there is no demonstrable change from the normal Where do they conceive this toxic substance to arise? They disprove Draper Maury's contention that it is a normal output of the mucosa, which is poisonous unless neutralized with fluids from other portions of the alimentary tract, by showing that duodenal loop fluid, digested with normal intestinal mucosa, loses none of its toxicity (p 316) With a normally secreting mucous membrane, undergoing no change from the normal, it is difficult to conceive how there is rapidly formed a very toxic material which is directly absorbed from it into the general circulation, with resulting death It seems inevitable that severe changes take place in the mucosa —they are usually demonstrable—and that the toxic material comes from this alteration in the mucosa In all cases in which death occurs from the closed loop itself some evidence of damage to the mucosa is described and the severity of the symptoms runs parallel to the severity of the demonstrable damage

A careful study, therefore, of the findings of these authors makes us unwilling to accept their conclusion that a poisonous substance is poured out by a normal mucosa, or elaborated and directly absorbed by the normal cells, which substance produces the toxic symptoms in simple high intestinal obstruction In discussing the fact that dogs with a simple obstruction may be kept alive at will by simply replacing with normal saline solution the fluid lost in the vomitus, Whipple, Stone and Bernheim say that the benefit derived "is due to dilution and elimination of the toxic material by this means, and the development of a gradually increasing immunity which can be shown to appear in dogs after intravenous injection of the toxic substance' (p 321) Yet they report conclusive experiments showing that their toxic substance neither exists in the blood of a closed loop dog nor remains for two hours in the blood of a dog that has received a lethal dose intravenously (p 309) It is difficult to comprehend, then, how saline solution given once a day is capable of washing out from the blood a substance which, within two or three hours, is "fixed by the cells or partly destroyed," and which, if not washed out, will inevitably cause death If this were possible, one ought to find a cure for organic poisons by a hypodermoclysis, and, similarly, the closed-loop dogs ought to be saved by this means

In commenting on the claim of Hartwell and Hoguet,[20] that this action of salt solution is a strong argument against an essential toxemia, and that the loss of water is the important factor, Whipple and his associates say "One might argue in the same manner that the appearance of sugar in the urine is the cause of diabetes" The analogy is incorrect Were it possible to alleviate diabetes by the subcutaneous injections of sugar, to the extent that obstruction may be alleviated by injections of saline solution, the analogy would be acceptable, the loss of sugar being the prime factor in one case, and the loss of water in the other

Moreover, the most potent argument of Hartwell and Hoguet in this connection—a normal starving metabolism when saline is given, and a completely deranged one when it is withheld, as determined by Benedict —is entirely ignored by Whipple, Stone and Bernheim

It is our contention that the experiments cited by these authors have no bearing on the subject of *uncomplicated* obstruction. They show, however, that a damaged intestine will produce a substance which is very poisonous if it gain access to the blood This is exactly the same conclusion we arrived at by a different method of investigation and reasoning

Still further to test the question, we produced the doubly closed loop in different parts of the bowel on a series of dogs

The experiments with a doubly occluded loop of intestine, made either by section and inversion of the ends, or by the clamps, need not be detailed because they duplicated in every way the results obtained by Whipple,

Stone and Bernheim It is worthy of note, however, that dogs with the double closure in the middle of the jejunum showed the same symptoms and died in the same way as those with a duodenal doubly closed loop When the jejunal loop became distended with its secretions, and the mucosal circulation was damaged, the animals became sick and ultimately died The course, however, was less acute This proved that death is not due to any specific action of the duodenal mucosa, as Whipple, Stone and Bernheim assume When, however, a double occlusion is produced in the lower ileum, or the cecal region, the animals are not sick, and they may live an indefinite time Similar results have been obtained by von Baracz [23] They are explained by the fact that this part of the intestine secretes very little fluid and consequently there is no distention, no damage to the mucosa, and no absorption of the poisons which higher up result from such a damage

A series of experiments performed by us with the object of preventing such a damage even in the duodenum gave important results We believed that if we could minimize the amount of fluids poured out into the duodenal loop and the operative damage to the bowel wall, we could longer retain an intact mucosa, and hence longer delay the fatal outcome To accomplish this the operation was done in two stages At the first stage the bowel was sectioned just below the pancreatic duct and the two ends closed by inversion, with as little operative damage as possible The continuity of the alimentary canal was then reestablished by a lateral anastomosis between the upper duodenal segment and the beginning of the jejunum In this way there existed a segment of the duodenum below the ducts which was closed at the upper end and drained by continuity directly into the jejunum The animal was allowed to recover completely from this operation during a period of two weeks He was then kept without food for four days, and the second stage operation performed At this time the lower end of the duodenum was sectioned at the site of the entero-enterostomy and the two ends closed, the same care being taken to avoid needless damage to the mucosa in doing this Thus, there resulted a doubly closed duodenal loop Following this operation the animals received no food or water by mouth, but were given saline subcutaneously This was done with the belief that absence of the stimulation from food would materially decrease the secretions in the closed loop, and thereby prevent its overdistention

In a series of dogs we were able to get a positive result in two in that with a double occlusion of the duodenum without visible damage to the mucosa death did not result as it usually does in the one stage operation with its inevitable damage to the mucosa

23 Von Baracz Arch f klin Chir, 1899, lviii, 121

Dog 107.—May 1, 1913 An entero enterostomy was done between the duodenum just below the pancreatic duct and the first part of the jejunum The bowel was then sectioned and the ends closed by inversion at the proximal end of the loop of duodenum thus formed Recovery from this operation was completed by May 3, after which he was regularly fed and seemed to be quite normal

May 12 to 16 All food withheld but water given freely

May 16 Abdomen again opened, and the distal end of the duodenal loop was sectioned near the anastomosis and the two ends closed by inversion and suture Thus there was formed a doubly occluded loop of the whole duodenum, while an entero enterostomy reestablished the intestinal patency Following this operation he was moderately sick for four or five days He vomited somewhat He was given all the water he would drink and no hypodermoclysis At the end of a week he seemed entirely normal, ate greedily and had no trouble in digesting food On the seventeenth day after the second operation, the abdomen was again opened The duodenal loop was found tightly closed at both ends and practically empty The mucosa was slightly congested, but otherwise quite normal

Dog 108.—The same procedure was followed as in the previous case Twenty-five days after the second operation the abdomen was opened and the loop examined In this case there had been a reestablishment of the lumen from the distal end of the loop into the intestine This had been possible because after sectioning the bowel, and inverting the two ends at the second operation, they had been fastened in apposition so as to prevent an intussusception It is not conceivable that this could have resulted under one week, so that the dog lived during this time in good health with a doubly occluded duodenum

In the whole series of doubly occluded duodena which we did, there was always a direct relation between the severity of the symptoms and the shortness of postoperative life on the one hand, and the amount of mucosal damage on the other

The two cases cited above we submit as giving final proof to our contention that there is no fatal poison arising from the duodenum when there is no damage to the mucosa

LOW OBSTRUCTION

Having demonstrated the causes of death in high intestinal obstruction we next turned our attention to a study of an obstruction lower down In the following, the obstruction was produced by clamp in lower ileum or at cecum, and treated with small amounts of normal saline solution

Dog 48.—Weight 13 5 kg Fed twenty-four hours before operation Clamp applied just above cecum

December 12 Vomiting large amount of bile stained fluid Given water by mouth and 200 c c subcutaneously Condition good

December 13 Drank water greedily, 200 c c saline subcutaneously Condition good Temperature, 40 Pulse, 120 Vomiting somewhat

December 14 Same

December 15 Condition not so good Acts sick Pulse 145

December 16 Very sick Vomitus excessive Bright red in color Fecal in character and very foul Pulse 180

December 17 Died during night Lived five and one half days

Autopsy.—Obstruction complete Small abscess around clamp No peritonitis Very intense congestion throughout peritoneum Kidneys and liver intensely congested

Alimentary Tract Stomach not distended, but mucosa somewhat congested Duodenum the same The entire ileum is greatly distended, wall thin, mucosa intensely congested In lower part hemorrhagic areas are present, and in spots superficial areas of necrosis exist

Damage to mucosa caused death

Dog 50.—Weight 5 kg Fed few hours before operation Clamp applied just above cecum

The postoperative condition exactly parallel to that of Dog 48 Remained in excellent condition for three days, then began to sicken and died after five and one half days He received from 150 to 210 c c water subcutaneously each day and drank in addition Vomited every day

Autopsy.—Obstruction complete No peritonitis Same changes found as in Dog 48 The ileum above the clamp was enormously distended and black in color No actual necrosis seen Kidneys and liver markedly congested

Dog 52.—Fed twenty four hours before operation Weight 12 kg Clamp applied just above cecum

Postoperative behavior as in previous cases, except that he remained well for nine days, after which he grew sick Received 200 c c of saline subcutaneously each day Vomiting much less than in other cases

Killed with ether on tenth day while in apparent fair condition

Autopsy.—Obstruction complete No pertitonitis Upper bowel empty Lower ileum much congested, markedly distended and containing a quantity of foul smelling liquid, fecal matter There was a marked peristalsis still present, and the fluid contents of the bowel could be seen continuously forced downward to the site of the obstruction There was no marked evidence of the damage to the intestinal wall seen in the other cases The liver showed some congestion, but otherwise the organs appeared normal [24]

Cultures from the peritoneum, femoral blood, spleen and liver all proved negative, both anaerobically and aerobically

This type of experiment was done six times, and the results accorded in every way with the three here described Length of life exactly paralleled the lack of injury to the intestine as it had done in the high obstruction

In the following low obstruction was treated with excess of saline subcutaneously

Dog 85.—Clamp applied 62 cm above the cecum Normal saline, 400 c c, was given subcutaneously at the end of the operation This was repeated the next day, but in spite of it the dog only lived forty six hours

Autopsy.—No peritonitis Obstruction complete The bowel below the clamp is collapsed and empty Above the clamp the bowel is dilated all the way to the stomach, being four or five times the normal diameter for a distance of 150 cm This intestine shows very marked vascular changes throughout this length, as instanced by the presence of deep ecchymoses which involve all the coats except the peritoneal, through which, however, they are readily seen

These changes are most marked on the convex border of the intestine, the surface at which the circulation would be naturally most disturbed by the excessive distention There are no actual ulcers or necrotic areas seen in the mucosa

Microscopical Examination.—The intestinal mucosa in the above areas is practically destroyed There is edema throughout the entire wall of the bowel

24 The length of life, and the absence of symptoms for such a long period, was due to lack of excessive vomiting and lack of damage to the intestine

Between all the layers is a beginning purulent exudate with hemorrhages These changes are probably of microbic origin

Dog 86—Clamp applied 40 cm above the cecum Dog remained perfectly well for three days On the fourth day he began to act sick, and on the fifth day was greatly prostrated though still able to walk around the laboratory During the five days the total output of urine and vomitus was 2,030 c c, which was replaced by the administration of 2 050 c c of normal saline solution The dog was killed with ether and the autopsy immediately done

Autopsy—No peritonitis The clamp was tight and produced a complete obstruction The bowel above the clamp was moderately distended for a distance of 50 cm, and shows no gross changes on peritoneal surface except for congestion immediately above the clamp

When the bowel is opened there are seen many scattered small areas where the mucosa is much damaged, in places showing a beginning necrosis The content of the bowel is ordinary fluid fecal material

Microscopical Examination—There is an almost complete necrotic destruction of the mucosa at the points mentioned above The entire thickness of the bowel wall is edematous and shows signs of acute exudative inflammation

Kidneys Acute parenchymatous inflammation is present

Liver Granular and lipoid degenerative changes are present

Dog 88—The clamp was applied 100 cm above the cecum This animal lived ten days, having remained in excellent condition for eight days The vomiting was not so excessive as in the high obstruction but was fecal in character and exceedingly offensive after the third or fourth day The total output of urine and vomitus in ten days was 2 030 c c and the saline injections amounted to 3,900 c c

Autopsy—There was no peritonitis The obstruction was complete, the bowel below the clamp being collapsed and empty Above the clamp the intestine was distended and filled with foul smelling fluid feces There was a slight purplish discoloration of the bowel for about 35 cm above the obstruction The mucosa appeared somewhat edematous, but there was no evidence of ulceration or necrosis

Microscopical Examination—The intestine shows congestion and edema There are areas in which some necrosis is present with exfoliation of the mucosal cells No pyogenic infiltration is seen

Liver shows only congestion

Kidney shows congestion and some parenchymatous degeneration

Dog 87—The clamp was applied in the lower ileum This animal lived in apparent perfect health for eighteen days, when he was killed The total output of urine and vomitus was 3,950 c c and the injections of normal saline totaled 6,600 c c

It will be noted that the output of fluids is far less than in the high obstructions, and hence the quantities of saline injected may be reduced

Autopsy—There was no peritonitis The obstruction was complete the bowel below being empty and collapsed The bowel above the clamp was moderately distended, but there was an absence of the discoloration, hemorrhages and other evidences of tissue damage seen in the other cases On opening the intestine the mucosa appeared absolutely normal throughout, except for one small area 5 cm in diameter, about 20 cm above the clamp, where there was an ecchymosis

Microscopical Examination—The intestine shows only a slight congestion There is an absence of the lesions found in the animals which died at an early date

The kidneys and liver are entirely normal

The study of low obstruction gives, therefore, results exactly like those found in the high obstruction Dogs may be kept alive indefinitely by the injection of an excess of normal saline solution, if the intestinal

wall remains undamaged Such injections, however, will not save life where there exist lesions in the mucosa which destroy the natural protection against the absorption of bacterial and other poisons from the lumen of the bowel

It may be asked what actual evidence has been found that poisons enter the system even under these conditions The evidence is indirect and inferential It is plain that so long as the mucosa remains intact, and desiccation of the tissues is prevented, there is no absorption of poisons because the animals remain in perfect health This does not mean that the stagnated intestinal contents would not prove poisonous if they could enter the blood On the contrary, they are poisonous even after sterilization by boiling and filtering, as many investigators have shown In every instance, however, in which serious symptoms of sickness develop and ultimately the animal dies, a damaged mucosa is found at autopsy Study of this lesion shows it to be a destructive one The mucosa is more or less destroyed and all the evidence is present that there is a bacterial inflammation in the intestinal wall The severity of the symptoms varies directly and the length of life inversely with the extent of this damage It is just as certain an inference that some poison is absorbed under these circumstances as any other of the many infective inflammatory lesions which we recognize as being the source of a toxemia, even though a bacteriemia is absent

There are two points to be explained in comparing the high and low obstruction First, why do the animals with the latter vomit a less quantity? Second, why cannot all the dogs with a low obstruction be kept alive by the administration of saline solution?

The vomitus is reduced in the low obstruction because there is a long surface of intestine through which absorption takes place Similarly the dogs are less desiccated because the water content of the bile, gastric and intestinal juices reenter the body On the other hand, there is practically no absorbing surface available for this purpose in the high obstructions, and the distance backward into the stomach being short, the latter organ rapidly fills and vomiting is imperative and almost incessant

The explanation of the inability to save life in low obstruction is found in the mechanical conditions which exist The small intestine above the obstruction becomes distended, and following this, kinking and tension on the mesentery develops In this way there are areas in which severe circulatory disturbance is present, resulting in the very changes which we have found will cause death and which are uninfluenced by the administration of saline subcutaneously

The results of our investigations of intestinal obstructions may be summarized as follows

1 There are two factors at work in producing the symptoms and causing death in this condition.

First, the loss of water from the tissues, due to the excessive drain into the bowel in response to the irritation set up by the retained secretions This water may be replaced by subcutaneous injection of normal saline solution, and the symptoms caused are thus entirely controlled, with a saving of life

Second, the entrance into the circulation of poisonous materials, which occurs only when the mucosa of the bowel is damaged This damage results largely from the trauma inflicted by the overdistention acting on the circulation, but possibly also by the chemical action of the digestive ferments stagnated above the obstruction Such a damage having resulted, there occurs a bacterial invasion into the bowel wall with a death of tissue cells (which is invariably demonstrable by microscopical examination), and in this process the poisonous substances are elaborated The action of these poisons is apparently not influenced by the administration of saline subcutaneously

The absorption of a poison from any source whatsoever, so long as the mucosa remains intact, is not a factor

2 The development of a bacteriemia as a cause of death is excluded

CLINICAL APPLICATION

It is not always safe to transfer the findings of experimental work to clinical practice, and as yet we have had no extended opportunity to apply the teaching of our investigations to the bedside There are certain applications, however, which seem justified It has always been a matter of speculation why high intestinal obstruction in man is so much more acute than low obstruction The explanation is found in the fact that water absorption is entirely stopped in the first, and in addition, enormous amounts of water are thrown out in the vomitus almost as soon as the obstruction occurs A rapid desiccation takes place, and with it a severely damaged metabolism, as shown by the deranged urine output These conditions obtain much more gradually when the obstruction is low in the bowel Another factor is also found in the sudden enormous duodenal and gastric distention which mechanically damages the mucosa

It is a familiar observation that a chronic obstruction may take place in the bowel and progress until the opening is only pin-point in size, yet no serious symptoms arise, even though there may be for many days cessation of fecal passage from the bowels When, however, a complete closure supervenes, there arises immediate evidence of a severe condition The well-known symptoms of acute intestinal obstruction are manifest The explanation of this is found in the fact that so long

as gas can escape through the constriction, there is no distention of the intestine, and hence no damage to the intestinal wall, and no absorption of poisonous materials The stoppage of gas, however, is immediately followed by both these conditions, and unless promptly relieved will result fatally The practical application of the principles elaborated by the experimental work follows two lines First, the water extracted from the tissues by the enormous outpouring into the bowel must be replaced This can easily be done by means of a continuous hypodermoclysis administered under the skin in the pectoral region by use of needles of the ordinary hypodermic caliber, but longer In this way the fluid is absorbed almost as rapidly as it enters, 1 or 2 liters being given without withdrawing the needles The absorption will continue until the normal water content of the tissues is reestablished Since, however, this has no influence on the symptoms resulting from a damaged intestinal wall, it is imperative to relieve the obstruction surgically as early as it is discovered A second point of practical importance is derived from our experiments, that is, an answer to the question as to the advisability of opening the bowel, and either temporarily or permanently draining off the contents Whenever there has resulted from the obstruction any considerable damage to the bowel wall above the constricted point, by reason of distention and circulatory changes, there is danger of serious symptoms arising from the absorption of materials resulting from these changes Therefore, in the presence of such changes, the more rapid emptying of the bowel through an enterostomy than through the normal path, after relieving the obstruction, may be a wise thing to do Whether the drainage of the intestine shall be made to continue after primarily emptying as much as may be possible of the distended bowel depends on the extent of the damage done If this is great in amount, it may be advisable to establish an enterostomy, because there will be some farther accumulation of the poisons which will be absorbed unless there is a free exit for them Such an enterostomy will be necessary if the contents of the bowel above the obstruction are blood-tinged, demonstrating a damage to the bowel wall If it is simply fluid feces, the rapidly returning peristalsis, after the obstruction is released, will force the contents onward, and there will be no danger of poisonous absorption when once it gets into normal bowel below the obstruction, because the mucosa here possesses a defense against such absorption

We desire to express our thanks to Drs Elser, Benedict, Ewing and Lusk of the Cornell University Medical College who gave us many valuable suggestions and material aid in parts of the work here presented

27 East Sixty-Third Street—40 East Forty First Street—55 East Sixty-First Street

THE CLASSIFICATION OF AMEBAS

WITH OBSERVATIONS ON MORPHOLOGY AND LIFE CYCLE OF ENTAMOEBA COLI, CRAIGIA HOMINIS AND VAHLKAMPFIA LOBOSPINOSA *

CHARLES F CRAIG, M D
FORT LEAVENWORTH, KAN

Recent observations on the morphology of the amebas encountered in the human intestine, and on their life cycle, have shown conclusively that there are two species of entamebas parasitic in the intestine of man, *Entamoeba histolytica* and *Entamoeba coli*, and that in rare instances species belonging to the genus *Vahlkampfia*, normally free living, may lead a semiparasitic existence in the human intestine for a longer or shorter period of time In view of these facts it has been necessary to revise our classification of amebas and in the following contribution I have adopted Calkins' classification and have recorded the latest observations on *E coli*, *Craigia hominis* and *Vahlkampfia lobospinosa* If some of the statements contained in the paper appear somewhat dogmatic I can only plead in excuse the fact that they are personal opinions based on years of careful study of the organisms in question

CLASSIFICATION AND NOMENCLATURE

It would be of little interest to describe the efforts made at the classification of the amebas occurring in man prior to the researches of Schaudinn In 1903[1] he divided these organisms into two species, *E histolytica* and *E coli* At present several classifications are advocated by different authorities and it is difficult to select that which is accepted by the greatest number of protozoologists The work of Musgrave and Clegg[2] on the cultivation of amebas from man early made it evident that amebas occur in the intestine and in the feces, as well as in the pus of liver abscesses, that were not identical with either *E histolytica* or *E coli*, and their conclusion that these cultivated amebas could cause dysentery resulted in the greatest confusion in classification and nomen-

* Submitted for publication March 3, 1914

* From the Department Laboratory, Central Department, U S Army

1 Schaudinn, F Untersuchungen ueber die Fortpflanzung einiger Rhizopoden, Arb a d k Gsndhtsamte, 1903, xix, 547

2 Musgrave, W E, and Clegg, M T Amebas Their Cultivation and Etiologic Significance, Bull Govt Lab, Biolog Lab, Manila, 1904, 18

clature The work of Hartmann[3] and Whitmore,[4] as well as that of many other investigators, in proving that these cultivated amebas are not entamebas at all and have nothing to do with the production of dysentery, has resulted in a clearer conception of the entire question of classification of the parasitic amebas, or entamebas, and their relation to infection and the production of disease

At present it is admitted that the old genus *Amoeba* should be split into several genera, some of these living free in nature, while others are either partly or wholly parasitic in their life habits It is also recognized that some of the free-living forms frequently contaminate the food of man and thus reach the intestinal canal, where they may be demonstrated in the feces, generally in the encysted stage of development, but sometimes as vegetative forms, and it is believed that certain of these free-living species may actually become temporary parasites of man or other animals The amebas that have been cultivated from the feces belong to these free-living species, and have either reached the feces through being swallowed in infected food or water, or by aerial contamination These species differ entirely from the entamebas both in morphology and life history Although under the action of certain stimulins in artificial culture-mediums these organisms may show developmental stages similar to those observed in entamebas, and their morphology may become markedly modified, as proved by Calkins and Williams,[5] a careful study of these modified organisms will show that, after all, the nuclear structure is quite different from that of either *E histolytica* or *E coli*, and that there is no reason to believe that the latter species ever are transformed into the free-living species That the genus *Vahlkampfia* contains species that can be cultivated from the intestine or feces of man is undoubted, and that under cultivation these species may undergo marked modifications in morphology and life history is true, but that they are identical with true entamebas is disproved by the observations of nearly all authorities who have worked with both genera

The modern classification of amebas has been attempted by many protozoologists, and Chatton,[6] Alexeieff,[7] Doflein,[8] and Hartmann[3] have each proposed classifications that possess advantages In this paper,

3 Hartmann, M Article "Die Dysenterie Amoeben" in Von Prowazek's Handbuch der Pathogenen Protozoen, 1912, i, 51

4 Whitmore, E R Parasitäre und Freilebende Amoeben aus Manila und Saigon, etc, Arch f Protistenk, 1911, xxiii, 71

5 Williams, Anna, and Calkins, G N Cultural Amoebae, a Study in Variation, Jour Med Research, 1913, xxix, 43

6 Chatton, E, and Lalung Bonnaire, P Amibe limax (Vahlkampfia n gen) dans l'intestin humaine, Bull Soc path exot, 1912, v, 13

7 Alexeieff, A Sur les caractères cytologiques et la systématique des amibes du groupe Limax, Bull Soc zool de France, 1912, v, 55

8 Doflein, F Lehrbuch der Protozoenkunde edit 3, 1911, p 560

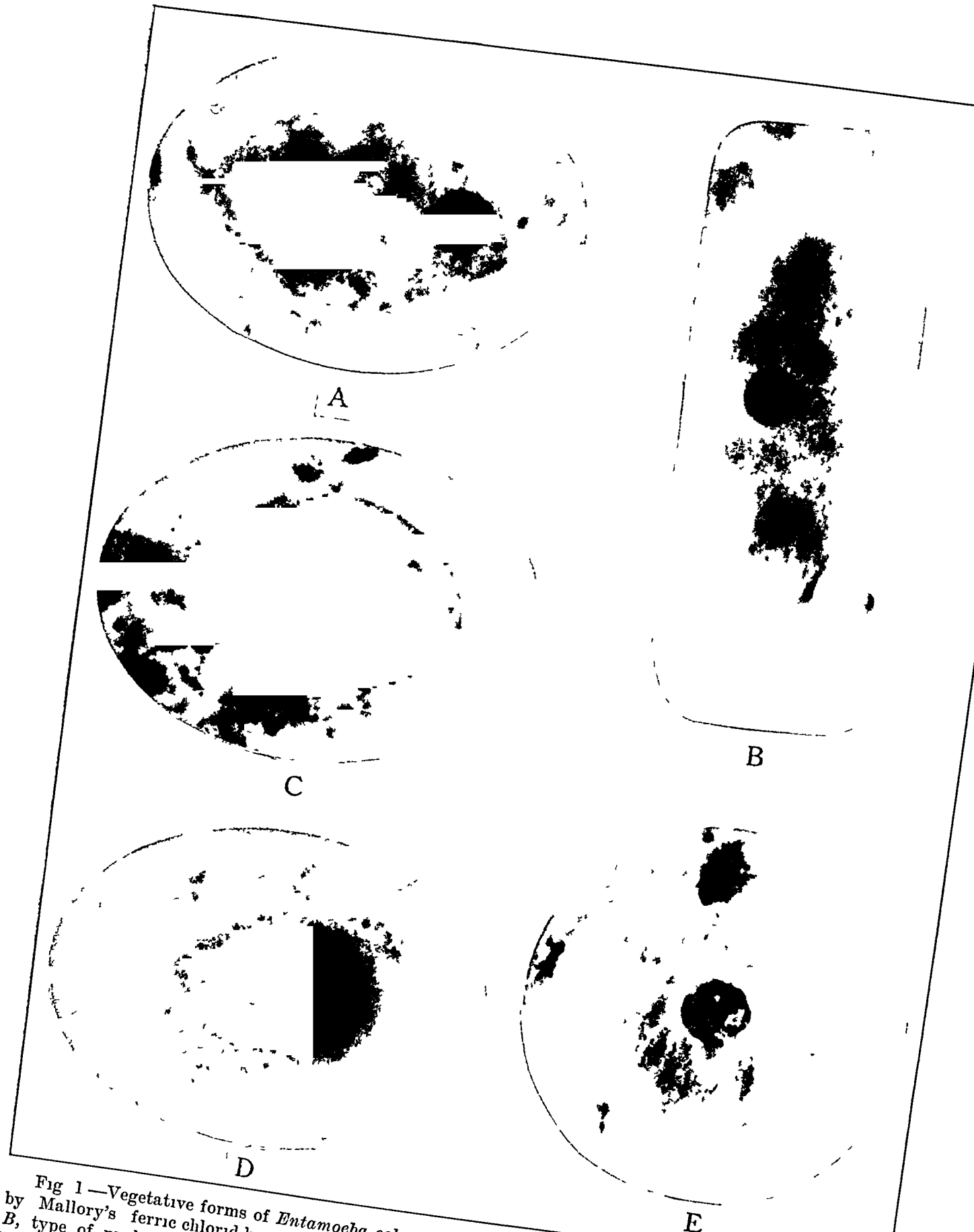


Fig 1.—Vegetative forms of *Entamoeba coli*, from photomicrographs × 1,200, all specimens stained by Mallory's ferric chlorid hematoxylin method *A*, large-sized organism with characteristic nucleus, *B*, type of nucleus before division begins, *C*, typical nucleus, *D*, nucleus with chromatin on inner border of nuclear membrane, *E*, typical karyosome, granular appearance of the nuclear membrane, linin net work and intranuclear chromatin

however, I shall follow the classification adopted by Calkins,[9] because I believe it to be the most logical at the present stage of our knowledge of the amebas It must be remembered, however, that any classification, at present, must be purely tentative until the life cycle of every organism contained in it is known, and this is true of only a very few of the so-called species of amebas It should, therefore, be clearly understood, that while I adopt for the present the classification of Calkins, and while I shall list all described species of amebas under their respective genera in this classification, I believe that in time it will have to be changed, and that many of the described species will eventually be found to be identical with those better known

Calkins divides the old genus *Amoeba* into seven genera, as follows *Amoeba, Vahlkampfia, Naglería, Paramoeba, Craigia, Trimastigamoeba,* and *Entamoeba* Of these seven genera the only ones of interest to us in our discussion of the parasitic amebas of man are *Vahlkampfia, Craigia, Trimastigamoeba* and *Entamoeba*

Genus *Vahlkampfia,* Chatton, 1912 Chatton established this genus in 1912, as containing certain small amebas originally classed in the genus *Amoeba* under the so-called limax type, and excluding organisms having a flagellate stage of existence

Amebas of this genus are characterized by the presence in each of one or more nuclei and one or more contractile vacuoles They divide by simple division or a primitive form of mitosis, forming a uninucleate cyst Under certain abnormal cultural conditions the cyst may contain more than one nucleus, and marked morphological changes may occur in the nucleus (Craig) The nucleus in the vegetative stage contains a very large karyosome which sometimes shows a distinct centriole Habitat water, earth, and occasionally in man or other animals

The following species are included in this genus

Vahlkampfia limax, Dujardin, 1841, habitat, fresh water
Vahlkampfia guttula Dujardin, 1841, habitat, pond water
Vahlkampfia polypodia, Schultz, 1875, habitat, pond water
Vahlkampfia binucleata, Gruber, 1884, habitat, pond water
Vahlkampfia diploidea Hartmann and Nagler, 1908, habitat, garden earth
Vahlkampfia albida, Nagler, 1909, habitat, garden earth
Vahlkampfia horticola, Nagler, 1909, habitat, garden earth
Vahlkampfia lacustris, Nagler, 1909, habitat, fresh water
Vahlkampfia froschi, Hartmann, 1907, habitat, fresh water
Vahlkampfia lacertae, Hartmann, 1907, habitat, in intestine of lizards
Vahlkampfia mucicola, Chatton, 1909, habitat, ectoparasitic on fish
Vahlkampfia paedophthora, Caullery, 1910, habitat, eggs and embryos, crabs
Vahlkampfia tachypodia, Glaser, 1912, habitat pond-water
Vahlkampfia lamellipodia, Glaser, 1912, habitat, pond-water
Vahlkampfia lobospinosa, Craig, 1912, habitat, ditch water
Vahlkampfia whitmori, Hartmann, 1913, habitat, swamp water

9 Calkins, G N Genera and Species of Amoeba, Tr Fifteenth Internat Cong Hyg and Demog, 1912, II, 287

While most of the species so far described in this genus are free living, there is excellent evidence on record that some of them may become parasitic under certain conditions Of those mentioned in the list, the one described by Hartmann as *Vahlkampfia lacertae* is certainly a parasitic species, as are *V mucicola* and *V paedophthora* Several observers have already noted the occurrence of typical *Vahlkampfiae* in the feces of man in both the vegetative and cystic stages of development, when the question of outside contamination could be eliminated I have seen some beautiful preparations containing a typical *Vahlkampfia* obtained by Dr W M James from the feces of a patient observed at Panama The organisms are in both the vegetative and cystic stages of development and were observed over a considerable period of time, so that the question of contamination of the feces was ruled out It is interesting to observe that these amebas were easily distinguishable from either *E histolytica* or *E coli* in both the vegetative and encysted stages of development

It is probable that nearly all species of *Vahlkampfia* may be cultivated on artificial mediums, and it is this fact that has given rise to so much confusion regarding the cultivation of the true parasitic amebas These organisms frequently contaminate fecal or other material derived from patients suffering from entamebic dysentery, and may thus be obtained in cultures Amebas belonging to this genus have been cultivated by many observers, notably Musgrave and Clegg,[2] in impure culture, and by Williams[10] in pure culture, but the claims made by these investigators that the amebas so cultivated are identical with the true entamebas are unsupported by any adequate evidence It is now admitted by all competent observers that neither *E histolytica* nor *E coli* has been cultivated

The *Vahlkampfia* named in the above list as *Vahlkampfia lobospinosa* is a true free-living ameba that was first cultivated by Musgrave and Clegg from the feces of a dysentery patient It has since been obtained in pure culture by Williams This organism, as shown by Calkins and Williams,[5] when growing in pure culture, undergoes marked morphological and cyclical changes in response to certain cultural conditions, but in the human feces it possesses the morphology of a true *Vahlkampfia,* and even in the cultures is not indistinguishable from the entamebas found in man, as is well shown in the photomicrographs accompanying the paper by these observers This species has been under cultivation for several years, and the cysts have been found to resist drying for over one year, a fact that alone proves it to be entirely distinct from the entamebas of the intestine of man, for all the evidence of recent researches

10 Williams, Anna Pure Cultures of Amoebae Found in Intestines of Animals, Studies Research Lab, New York Board of Health, 1912, vi, 298

proves that the cysts of these entamebas have comparatively little resistance

While, as has been stated, species belonging to this genus have been observed apparently parasitic in man, and while, in some cases, they may be cultivated from the feces, the pus of liver abscesses, and other sources, there is no evidence that demonstrates that they are pathogenic or that they can live for any considerable time within the human intestine In the vast majority of instances they reach the material from which they are cultivated from the air, as proved by Liston[11] and Wells,[12] or by passing through the intestinal canal in contaminated food or water, generally in the encysted condition It is true that, in rare instances, species of *Vahlkampfia* may become parasitic for a short time in the human intestine, but this is really an abnormal mode of existence, no symptoms of disease are produced, and they may be easily distinguished from the true entamebas by the large nuclear karyosome, the uninucleate cyst, and the presence of at least one contractile vacuole Their presence in the intestine is chiefly of interest from the point of view of evolution, as indicating the possible origin of the true parasitic amebas

Genus *Craigia,* Calkins, 1912 In 1912 Calkins[9] established the genus *Craigia* to include the ameba described by myself under the name *Paramoeba hominis,* in 1906 This ameba was originally placed in the genus *Paramoeba* because of its resemblance to Schaudinn's *Paramoeba eilhardi,* but Calkins has shown that it should be placed in a new genus, an opinion previously expressed by Doflein [8]

The type-species of this genus is *Craigia hominis* (*Paramoeba hominis*) a parasitic ameba that I have observed in twelve patients suffering from a chronic form of diarrhea It is characterized by a nucleus, an accessory nuclear body, and by an ameboid and flagellate stage of development Multiplication occurs in the ameboid stage by simple division and by the formation of cysts containing numerous minute flagellated organisms In the flagellate stage of development multiplication occurs by longitudinal division, preceded by division of the accessory body and the nucleus Habitat, the intestine of man

The following species are included in this genus

Craigia hominis, Craig, 1906, emend Calkins, 1912
Craigia pigmentifera, Grassi, 1881, habitat, body cavity of *Chaetognathi*
Craigia chaetognathi, Grassi, 1881, habitat, body cavity of *Chaetognathi*

It is interesting to note that all of the species so far described for this genus are parasitic, *Craigia hominis* being parasitic in man, while

11 Liston, W Glen A Note on Early Stages of Nuclear Division, Quart Jour Micr Sc, 1911, lvii, 279

12 Wells, R T Aerial Contaminations as a Fallacy in the Study of Amoebic Infections by Cultural Methods, Parasitology, 1911, iv, 204

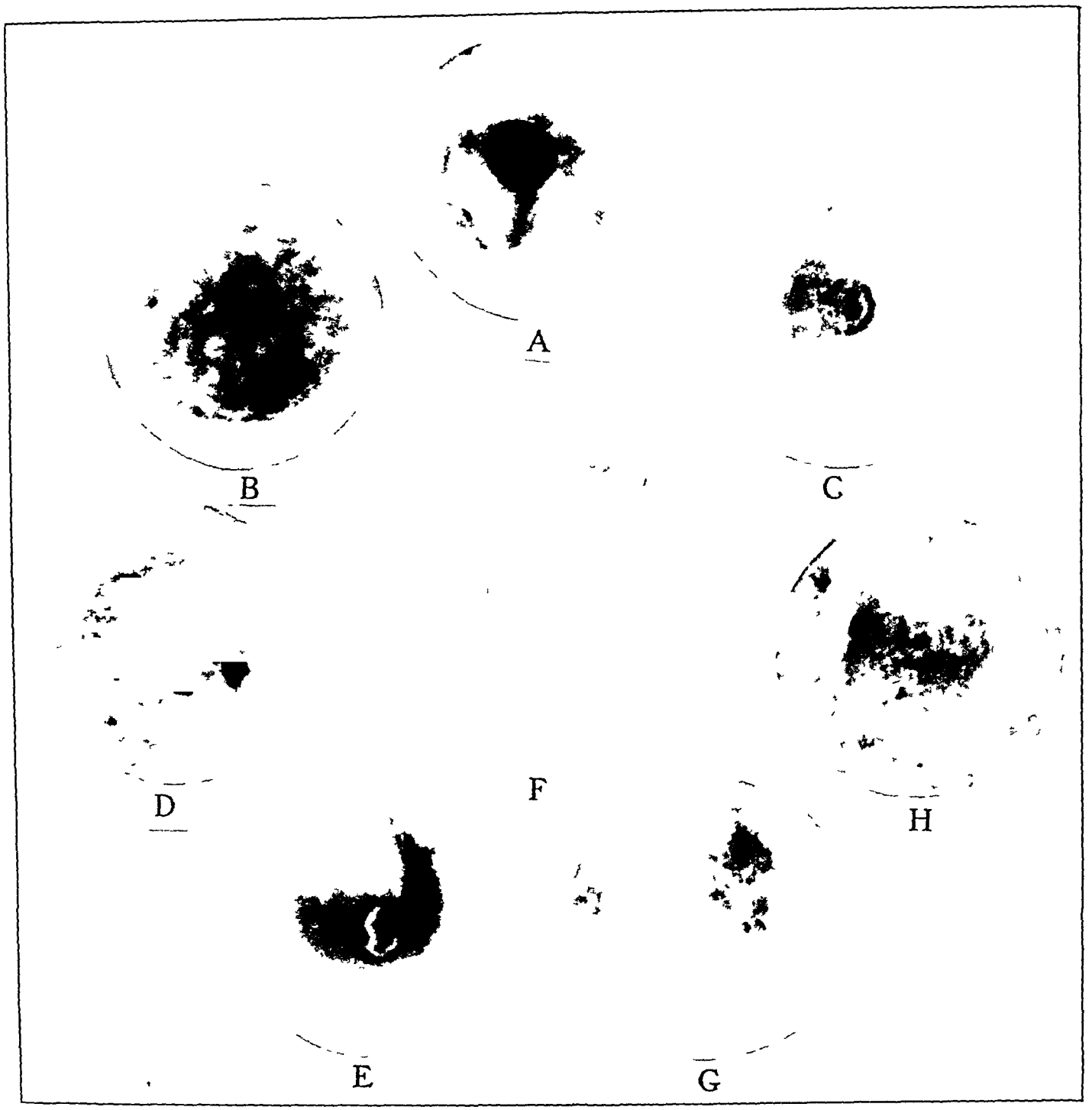


Fig 2.—Precystic and cystic form of *Entameba coli*, from photomicrographs × 1,200, *A*, precystic form in which the nucleus is more than usually typical, *B*, division of the karyosome into two portions, *C*, precystic form showing change in type of nucleus and slight elongation of karyosome, *D*, precystic form containing yeast cells, *E*, typical cyst showing nucleus in which the karyosome has apparently divided, *F*, very large cyst showing large vacuole and nucleus in which the karyosome has elongated preparatory to division, *G*, cyst containing six nuclei, eight were present but two are out of focus, *H*, cyst containing three definite nuclei Four were present, but one does not show as it lies out of focus

Craigia pigmentifera and *Craigia chaetognathi* are both parasitic in certain species of marine worms

This genus is of considerable interest to the physician, as it contains a species which, during the ameboid stage, may be easily confused, by one unfamiliar with the protozoa of the intestine, with amebas belonging to the genus *Vahlkampfia* or *Entamoeba,* and in the flagellate stage with *Trichomonas hominis* or other intestinal flagellates This mistake, however, could never be made by any one well acquainted with the morphology of the parasites mentioned

Genus *Trimastigamoeba,* Whitmore, 1911 Whitmore[13] established this genus, in 1911, to include an ameba isolated by himself in cultures from tap-water, in Manila The type-species is *Trimastigamoeba philippinensis,* and is characterized by a nucleus of the limax type, a contractile vacuole, and the occurrence, under certain conditions, of flagellate forms having three equal flagella The species is easily cultivated on suitable mediums, and is the only one so far placed in the genus This ameba is of interest to physicians, because it may contaminate fecal or other material from human sources and appear in cultures prepared from such material There is no evidence that this species occurs as a parasite in man or other animals

Genus *Entamoeba,* Leidy, 1879 This genus, which contains most of the parasitic amebas of man and other animals, was established in 1879 by Joseph Leidy,[4] who used the spelling *Endamoeba* in the description of the genus The type-species is *Entamoeba blattae,* a parasite of the cockroach Casagrandi and Barbagallo,[15] in 1897, unaware of Leidy's genus *Endamoeba,* established for the parasitic amebas the genus *Entamoeba,* and this is the spelling employed at present

The generic characters are as follows Size comparatively small, from 5 to 80 microns Ectoplasm and endoplasm distinct when the organisms are moving and often when motionless, contractile vacuole generally absent, nucleus with definite karyosome and centriole; nuclear division mesomitotic and mitotic, reproduction by simple division, schizogony, and cyst formation with the production of several amebas within the cyst Habitat, the body of man or other animals, generally in the digestive tract The genus contains only parasitic species, some of which are pathogenic, but most live merely as commensals in the intestine of their host None of the species have been artificially cultivated

13 Whitmore, E R Studien uber Kulturamoeben aus Manila, Arch f Protistenk, 1911, xxiii, 81

14 Leidy, Joseph Fresh Water Rhizopods of North America, Rep U S Geol Surv, Washington, 1879, xii, 1

15 Casagrandi, O G, and Barbagallo, P Ricerche biologiche e cliniche sull' Amoeba coli, Boll d Accad Gioenia di sc nat in Catalonia, n s, 1895, xxxix, 4

A very large number of species of amebas have been placed in this genus, and new species are being discovered almost monthly, but, as I have already stated, further study will prove many of these to be identical with species already studied I have placed in the list that follows, however, all the species reports of which I have been able to collect in the literature, but I have indicated those identified with other species The list is arranged alphabetically, and the name of the host follows that of the parasite

Entamoeba africana, Hartmann, 1907, identical with *Entamoeba histolytica*, host, man
Entamoeba blattae, Leidy 1879, host, cockroach
Entamoeba aulastomi, Noller, 1912, host, eagle
Entamoeba bovis, Liebetanz, 1905, host, cattle
Entamoeba brasilicnsis, Baurepaire Aragao, 1912, host, man
Entamoeba buccalis von Prowazek, 1907, host, man
Entamoeba butschlii, von Prowazek 1912, host, man
Entamoeba cobayae, Walker, 1908, host, guinea-pig
Entamoeba coli, Loesch, emend Schaudinn 1903, host man
Entamoeba dentalis, Braun, 1883, identical with *E buccalis*, host, man
Entamoeba duboscqi, Mathias, 1913 emend Craig, 1913, host, monkey
Entamoeba dysenteriae Councilman and Lafleur, emend Craig, 1905, identical with *E histolytica*, host, man
Entamoeba gingivalis, Braun, 1895, identical with E buccalis, host, man
Entamoeba hartmanni, von Prowazek, 1912 host, man
Entamoeba histolytica, Schaudinn, 1903, host, man
Entamoeba intestinalis, Walker, 1908, host, cat
Entamoeba kartulisi, Doflein, 1901, host, man
Entamoeba lacertae, Hartmann, 1907, identical with *Lahlkampfia lacertae*, host, lizard
Entamoeba lagopidis, Fantham 1910, host, moor hen
Entamoeba legeri, Mathias, 1913, emend Craig, 1914, host, monkey
Entamoeba maxillaris, Kartulis, 1906 identical with *E kartulisi*, host, man
Entamoeba minuta, Elmassian, 1909, identical with *E histolytica*, host, man
Entamoeba minuta, Ijima, 1898 host, man
Entamoeba mortinatalium, Smith and Weidman, 1910, host, man
Entamoeba muris, Grassi, 1881, host, mouse
Entamoeba nipponica, Koidzumi, 1909, identical with *E coli*, host, man
Entamoeba nuttalli, Castellani, 1908 host, monkey
Entamoeba phagocytoides, Gauducheau, 1907, host, man
Entamoeba pitheci, von Prowazek, 1912, host, monkey
Entamoeba polecki, von Prowazek, 1912, host, swine and man
Entamoeba pulmonalis, Artault, 1898, host, man
Entamoeba ranarum, Grassi, 1879, host, frog
Entamoeba salpae Alexeieff, 1912, host, fish
Entamoeba schaudinni, Lesage, 1908, host, man
Entamoeba suis, Hartmann, host, swine
Entamoeba testudinis, Hartmann, 1910, host, turtle
Entamoeba tetragena, Viereck, 1907, identical with *E. histolytica*, host, man
Entamoeba tropicalis, Lesage, 1908, identical with *E coli*, host, man
Entamoeba undulans, Castellani, 1904, host, man
Entamoeba urogenitalis, Baelz, 1883, host, man
Entamoeba williamsi, von Prowazek, 1911, host, man

It is more than probable that at least two-thirds of these species here given as parasitic in man are not valid species, but, as I have not had the opportunity of studying them, I include in the list all of the species

of entamebas that have been described Of these, however, there are really only two species that are of great interest to the physician, that is, *E histolytica* and *E coli*, the former is the common pathogenic species causing entamebic dysentery and liver abscess in man, while the latter is a harmless commensal, found in a varying, but usually large proportion of healthy, as well as diseased individuals, in almost every part of the world

A word of explanation is necessary in regard to the classification of the so-called *E tetragena* This entameba was first described by Viereck,[16] in 1907, as a distinct species causing dysentery in man, and his observations were quickly confirmed by Hartmann[3] and Prowazek, Whitmore,[17] and myself,[18] but recent researches have proved conclusively that this species is really identical with *E histolytica*, a portion of the life cycle of the latter species having been erroneously described by Schaudinn and his followers, and first correctly described by Viereck in his description of *E tetragena* It therefore follows that *tetragena* ceases to exist as a distinct species and the name must be eliminated from our nomenclature

It has also been shown that *E minuta* is identical with *histolytica* and that *E nipponica* and *E tropicalis* are identical with *E coli*, so that these three names also should be eliminated as specific names of entamebas

Since the discovery that *E tetragena* was identical with *E histolytica*, the credit for which belongs to Walker,[19] nearly all students of the subject who have had sufficient material on which to work are agreed that two well-defined species of entamebas occur in the intestine of man Since 1905 I have consistently maintained the specificity of these two entamebas, that is, *E histolytica* and *E coli*, and in my first paper,[20] and since have insisted that it is possible to differentiate them in practically every case of infection Many observers have confirmed my conclusions, but the recent work of Walker and Sellards[21] has conclusively proved my statements regarding the pathogenic nature of *histolytica* and the non-pathogenicity of *coli*, and also my conclusions regarding the lack of identity of cultural amebas and those parasitic in man By experi-

16 Viereck, H Studien uber die in den Tropen erworbene Dysenterie, Arch f Schiffs u Tropen Hyg, 1907, ii, 1

17 Whitmore, E R Parasitaire und freilebende Amoeben aus Manila und Saigon, Arch f Protistenk, 1911, xxiii, 71

18 Craig, Charles F Entamoeba Tetragena as a Cause of Dysentery in the Philippine Islands, THE ARCHIVES INT MED, 1911, vii, 362

19 Walker, E L A Comparative Study of the Amoebae of the Manila Water Supply, etc, Philippine Jour Sc, Med Sec, 1911, vi, 259

20 Craig, Charles F Observations Upon Amebas Infecting the Human Intestine, with a Description of Two Species, Entamoeba Coli and Entamoeba Dysenteriae, Am Med, Philadelphia, 1905, ix, 854, 897 and 936

21 Walker, E L, and Sellards, A W Experimental Entamoebic Dysentery, Philippine Jour of Sc, Sec B, Trop Med, 1913, viii, 253

ments on man these authors have proved that the amebas that can be cultivated from water, from the feces of man and other animals, and from other sources, are incapable, when ingested, of producing lesions in man, and that they are not entamebas but free-living amebas which do not produce dysentery, while, as already stated, their experiments prove

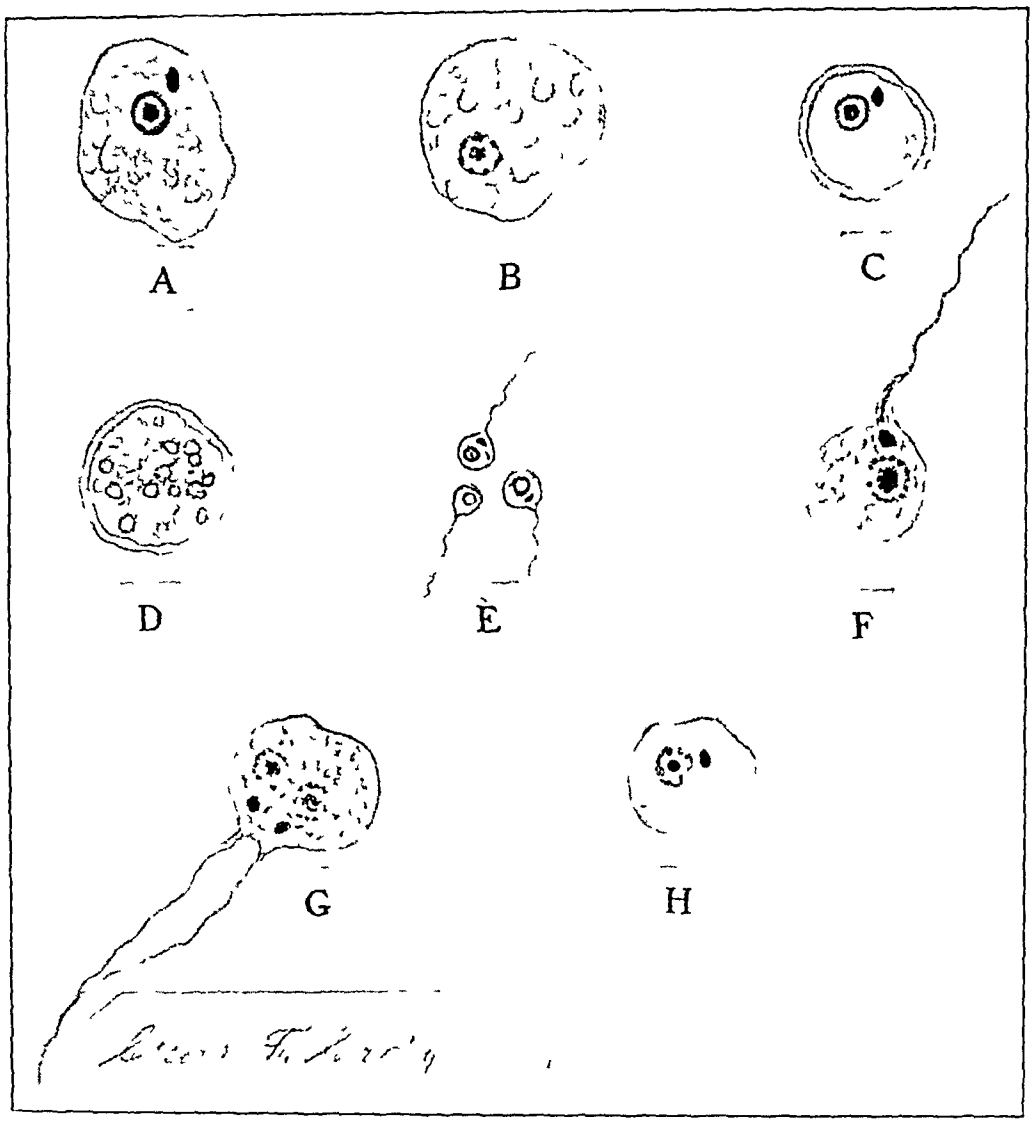


Fig 3.—*Craigia hominis*, from pencil drawings, × 1,200 *A*, typical organism in amebic stage (note structure of nucleus and accessory nuclear body), *B*, absence of accessory nuclear body and different type of nucleus, *C*, cyst showing one nucleus, *D*, cyst showing granular appearance of cytoplasm and the presence of numerous nuclei, *E*, young flagellate form, F, fully developed flagellate form (Note absence of undulating membane, the single flagellum, the nucleus, and accessory nuclear body), *G*, dividing flagellated form the flagellum being partly divided, while the nucleus and accessory nuclear body have divided, *H*, flagellate form after loss of flagellum and just before becoming ameboid

that *E histolytica* produces dysentery in man, and *E coli* is a harmless commensal in the human intestine While all of the essential points covered in Walker and Sellard's work have been previously proved by

experiments on the lower animals, their results possess the very great advantage of having been worked out on man, the natural host of *histolytica* and *coli,* and, in my opinion, settle for all time the question of the relation of parasitic and cultural amebas to disease in man

At present a considerable amount of controversy has arisen regarding the proper name for the common dysentery-producing entameba, usually known as *E histolytica* The name *tetragena* must be dropped as a specific name, although it may be used to designate the type of nucleus present during certain developmental stages of *histolytica* This conclusion is admitted by nearly all protozoologists, but Doflein and Hartmann favor substituting the specific name *dysenteriae* for *histolytica,* this name having been first proposed for the dysentery entameba by Councilman and Lafleur This is not a new proposition, for in 1905[20] I suggested that the name *dysenteriae* be given this entameba, but, as Stiles later showed that this name was merely a synonym of *coli,* and as Schaudinn had already used *coli* as the specific name for the harmless entameba of man, I adopted his name, *E histolytica,* for the pathogenic species

While it is undoubtedly true that Councilman and Lafleur described *E histolytica* in part in their exhaustive work on entamebic dysentery, it is perfectly evident to any one who studies their description carefully that they confused this parasite with the harmless *E coli,* and failed to differentiate between the pathogenic and harmless entamebas or to give morphological data sufficient to enable such a differentiation to be made by other workers In view of these facts and the fact that Schaudinn *did* differentiate the two species mentioned, I am convinced that, if we are to be consistent in our nomenclatural rules, the name *E histolytica,* given by him to the pathogenic species, must be retained The name *dysenteriae* is a much more descriptive and appropriate name, but if we are to be true to the accepted rules of nomenclature it cannot justly be applied to the entameba now known as *E histolytica*

OBSERVATION ON E COLI, LOESCH, 1875, EMEND SCHAUDINN, 1903

It was long known that amebas were frequently present in the feces of healthy persons and in those of patients suffering from other diseases than dysentery, Grassi[22] being one of the first to describe these amebas His work was followed by that of Calandruccio,[23] Schuberg,[24] Strong and

22 Grassi, G B Dei Protozoi parasiti e specialmente di quelli che sons nell' uomo, Gaz Med ital, Lombardie, 1879, xxxix, 445

23 Calandruccio, S Animali parasiti dell' uomo in Sicilia, Atti d Accad Gioenia di sc nat in Catania, 1889, lxvi, 95

24 Schuberg, A Die parasitischen Amoeben des menschlichen Darmes, Centralbl f Bakteriol, 1893, xiii, 598, 654 and 701

Musgrave,[25] and Casagrandi and Barbagallo[15] In 1903, Schaudinn[1] accurately described this entameba, and his observations were confirmed by myself in 1905[26] Important papers dealing with this parasite have since appeared by Werner[27] and Hartmann and Whitmore[28]

Several observers since Schaudinn have described as new species entamebas occurring in healthy, as well as diseased individuals, that answer closely to the description of *E coli* Among these may be mentioned *E tropicalis,* described by Lesage[29] in 1908, *E nipponica,* described by Koidzumi[30] in 1909, and *E williamsi,* described by von Prowazek[31] in 1911 Recent observations have shown that all these so-called species are identical with *E coli,* and it is probable that some other species of entamebas that have been described in the intestine of man are also identical with this parasite

The recognition of this species is of great importance because of its world-wide distribution, the fact that it is a harmless parasite, and the possibility of confusing it with the pathogenic *E histolytica* Our statistics of the actual occurrence of entamebic dysentery in all parts of the world are greatly vitiated by the non-recognition of this harmless species, many authorities making a diagnosis of entamebic dysentery whenever an ameba was found in the stools, without regard to the species present It is now evident that a diagnosis of entamebic dysentery based on anything less than the recognition of *E histolytica* in the feces cannot be considered as authentic, and if this test had been applied to the diagnosis of the disease in the past it is probable that our conceptions of its frequency in certain localities would be greatly modified

E coli has a very wide geographical distribution, being most frequently observed in tropical and subtropical regions, but being by no means infrequent in the temperate zones In discussing the relation of this organism to disease the distribution will be more fully considered, but it may be stated here that it has been found wherever determined efforts have been made to demonstrate it, and this fact makes its recognition of prime importance in the discussion of entamebic infection

25 Strong, R T, and Musgrave, W E Preliminary Note Regarding the Etiology of the Dysenteries of Manila, Rep Surg Gen U S Army, 1900, p 251

26 Craig, Charles F Observations Upon Amoebae Infecting the Human Intestine, Etc, Am Med, Philadelphia, 1905, ix, 854, 897 and 936

27 Werner, H Entamoeba Coli, Handbuch der path Protozoen, von Prowazek, i, 67

28 Hartmann, M, and Whitmore, E R Untersuchungen über Parasitische Amoeben Entamoeba coli, Arch f Protistenk, 1912, xxiv, 182

29 Lesage, A Culture de l'amibe de la dysenterie des pays chauds, Ann de l'Inst Pasteur, 1909, xviii, 9, Bull Soc de path Exot, 1908, v, 104

30 Koidzumi, M On a New Parasitic Amoeba, Entamoeba Nipponica, found in the Intestine of Japanese, Centralbl f Bakteriol, Orig, 1909, li, 650

31 Von Prowazek, S Beitrage zur Entamoeba Frage (Entamoeba williamsi), Arch f Protistenk, 1911, xxii, 345

Morphology.—Like *E histolytica*, this species varies greatly in morphology at different stages in its life cycle, and this cycle may also be divided into the vegetative stage, the precystic stage and the cystic stage, in each of which a description of the special morphology is necessary.

Morphology in the Vegetative Stage.—In this stage of development *E coli* varies greatly in size, very large and very small organisms being frequently observed in the same preparation The living vegetative parasites vary from 15 to 50 microns in diameter, and rarely may be larger, but the average diameter is about 25 to 35 microns The cytoplasm is divided into two portions, the ectoplasm and endoplasm, but the distinction is not possible save when the organisms are moving In this species the ectoplasm does not have the glass-like appearance of the ectoplasm of *E histolytica*, but approaches much more closely to the endoplasm in appearance, so that even when moving the ectoplasm is not nearly as distinct in *coli* as it is in *histolytica*, a very valuable differential point The *cytoplasm* contains a visible nucleus, appearing as a spherical body bounded by a refractile granular membrane and containing granules or small masses of refractile chromatin, generally arranged as a well-marked karyosome The nucleus of this species, even in the living condition, appears larger than does the *tetragena* type of nucleus of *E histolytica*, and this is more easily demonstrated in the living parasite The endoplasm appears very granular and contains numerous vacuoles, bacteria, and crystals taken up from the feces In contradistinction to *E histolytica*, this parasite is not phagocytic for red blood-corpuscles, even when the feces contain much blood, nor have I been able to demonstrate this phenomenon by adding fresh blood to feces containing *E coli*, although phagocytosis of the blood-corpuscles may often be observed if blood be added to fecal material containing *E histolytica* I have observed very rarely an entameba of this species with one or two red blood-corpuscles within the endoplasm, but I have never observed them filled with erythrocytes as in the case of *histolytica*, and I believe that when this process does occur in *coli* it is purely accidental, while in *histolytica* it is a normal process in the nutrition of the organism At any rate, the point is of great value in the differentiation of the two species, for one is quite safe in regarding any entameba containing red blood-corpuscles as a pathogenic parasite, presumably *E histolytica*

The motility of *E coli*, in the vegetative stage, is much less marked than that of *E histolytica*, although it may be quite active in the larger organisms in freshly voided feces To one who has had experience in the study of living specimens of both species, the difference in motility is very marked, *E coli*, even when most active, being sluggish in comparison with the average vegetative specimen of *E histolytica* The movement is also less progressive in character and more apt to be indefinite in direction

In stained preparations, (wet fixation and iron-haematoxylin) during the vegetative stage of development, the cytoplasm stains a brownish or grayish color and appears granular in consistence, containing bacteria, crystals, and generally a considerable number of vacuoles, but the most striking object is the nucleus, which in this species is much larger than either the histolytica or tetragena type of nucleus of *E histolytica,* in the vast majority of organisms examined, and much richer in chromatin The nucleus is spherical and situated

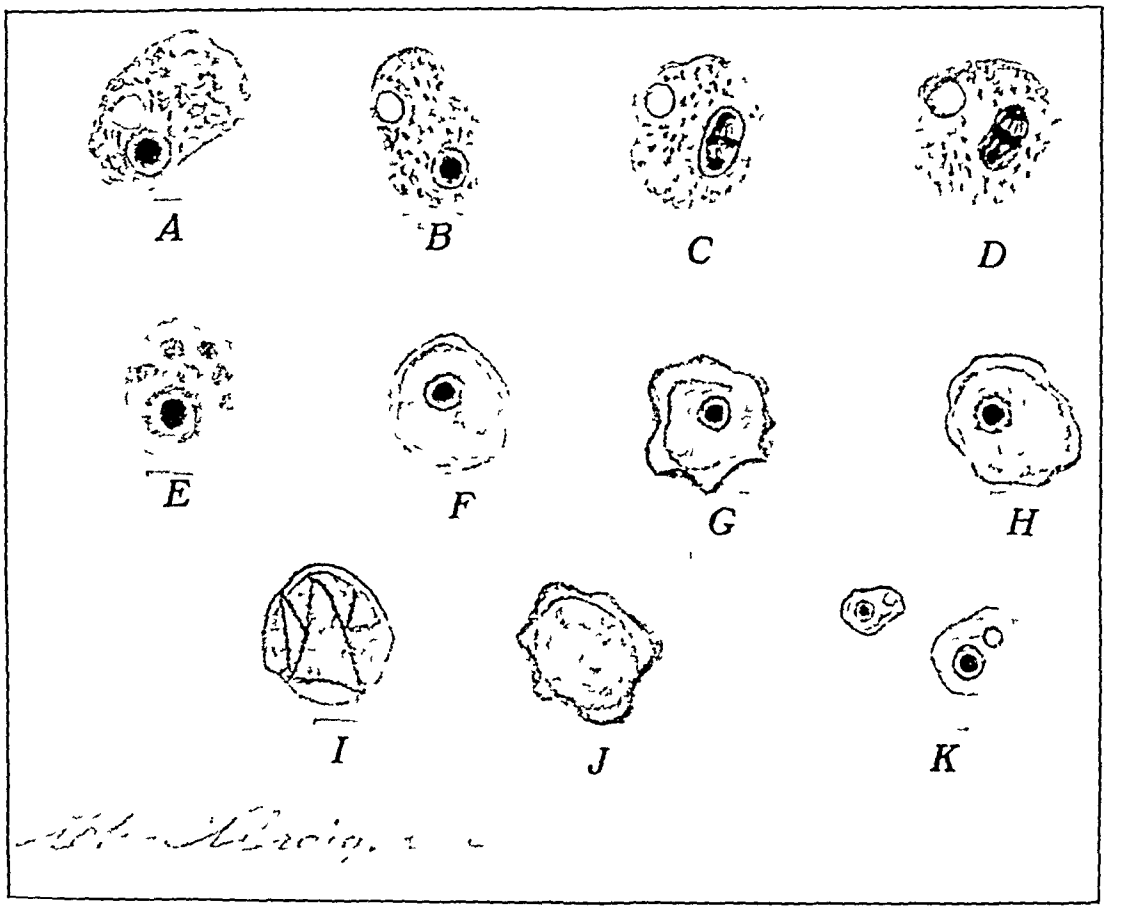


Fig 4—Vegetative forms of *Vahlkampfia lobospinosa,* from pencil drawings, × 1,200, *A,* shows large contractile vacuole near the nucleus, and typical nucleus (note the large, deeply staining karyosome), *B,* the contractile vacuole near the periphery of the cytoplasm, *C,* division of the nucleus, note the polar bodies and equatorial plate, *D,* nuclear division, *E,* organism containing yeast cells, note the granular appearance of the nuclear membrane, *F,* typical young cyst, *G,* old cyst, note thick cyst wall which is folded in places, a very characteristic appearance of the old cysts, *H, I, J,* cysts, *I* and *J,* degenerated, the nucleus having disappeared, *K,* young organisms shortly after liberation from cyst

within the endoplasm The nuclear membrane is thick and stains intensely black with hematoxylin It is even thicker than the membrane of the tetragena type of nucleus in *E histolytica* and presents on its inner surface granules, or more frequently, clumps of chromatin The

karyosome is situated at or near the center of the nucleus and appears most frequently as a spherical mass of chromatin, stained almost black, and containing no well-defined centriole In some instances a centriole may be distinguished as a more deeply staining dot of chromatin near the center of the karyosome In this species a certain series of cyclical changes occur in the nucleus, but they are not so pronounced as in the nucleus of *E histolytica,* when the tetragena type is present It is noted that when there is a large, spherical, solid-appearing karyosome there is little evidence of a linin network or of free chromatin within the nucleus, while the inner surface of the nuclear membrane is almost free from granules of chromatin, but when division of the karyosome occurs, this portion of the nucleus appears smaller and stains less intensely, while the linin network is more distinct, contains much chromatin in the form of small granules, and large clumps of this substance may be observed on the inner surface of the nuclear membrane The large clumps of chromatin just mentioned are quite characteristic of this species, for I have never observed such large masses on the nuclear membranes of the pathogenic species

Not infrequently specimens of *E coli* are observed undergoing simple division, and in stained preparations it is possible to study this process from the initial division of the centriole to the complete division of the nucleus Very frequently organisms are observed in which the karyosome consists of two deeply stained oval masses of chromatin situated near the center of the nucleus or near its periphery, these masses sometimes being connected by a dimly stained thread of chromatic substance More frequently the masses are apparently separate and either oval or spindle-shaped, while the interval between them appears dimly granular, but I have never observed any evidences of an equatorial plate Other organisms are observed in which the karyosome has disappeared and the inner surface of the nuclear membrane is covered with large, irregular clumps of chromatin, the remainder of the nucleus being filled with fine, dimly staining granules, and still others are observed containing two well-developed nuclei, each showing the large, spherical, deeply stained karyosome characteristic of the nucleus of this species before the changes prior to division occur

Besides the reproduction of the organism by division, Schaudinn described, in the vegetative stage of development of *E coli,* a process of schizogony, in which the parent entameba produced eight young entamebas I confirmed this observation and Hartmann and Whitmore[28] have published beautiful illustrations of vegetative organisms containing as many as six nuclei In my experience, this method of reproduction is very rarely observed, but I am convinced that it occurs and that the organisms in which I have observed it were not in the cystic stage of

development The process is initiated by an enlargement of the nucleus and a considerable increase in the amount of chromatin within it, the karyosome being indistinguishable Later the inner border of the nuclear membrane shows hemispheral masses of chromatin, arranged in a very regular manner, the remainder of the nucleus being entirely free of this substance. Organisms are observed containing from two to eight nuclei, each nucleus having a distinct nuclear membrane and a small, dot-like or granular appearing karyosome This method of reproduction has been questioned by several observers, notably James, but, in my opinion, no other interpretation can be placed on these organisms

Degenerative forms of *E coli* are frequently encountered during the vegetative stage of development, which may cause confusion in the diagnosis of the parasite In such organisms the nucleus may be invisible or distorted in appearance, and the cytoplasm may contain chromidia, as in *E histolytica* These organisms should not be considered in making a diagnosis of the species of entameba present, for typical parasites will always be found in stained preparations if carefully searched for, and the technic of staining has been correct

In some preparations entamebas are observed without a nucleus and free nuclei may be present in large numbers These free nuclei stain well and are typical in structure, and the exact significance of their occurrence is still in question This peculiar phenomenon was first called to my attention by James, who sent me preparations containing many examples of anucleate entamebas and free nuclei, and I have no explanation to offer of their presence, beyond the suggestion that possibly they may be due to some change brought about in fixing the preparation or in smearing it on the slide I do not believe that the condition is due to degenerative changes, for the nuclei appear normal as do the anucleate entamebas

The vegetative forms of *E coli* occur most typically in diarrheal stools or stools rendered liquid by some cathartic and, as this parasite is not pathogenic in character, these forms are seldom observed in the normal formed stools of the host, only the precystic or cystic forms being found in semiformed or formed stools For this reason I have found that the administration of a cathartic is essential if one desires to study the typical vegetative stage of *E coli,* but inadvisable if one desires to study the precystic or cystic stage It is unfortunate that the differentiation of *E coli* is of the greatest practical importance in just the class of cases in which the vegetative forms occur, that is, in cases showing diarrhea, for the vegetative forms are much harder to differentiate from the vegetative forms of *E histolytica* than are the cysts of the two species

Morphology in the Pre-Cystic Stage.—In semifluid stools the precystic forms of *E coli* are most frequently encountered. In this stage of development the parasite is reduced in size and resembles so closely the precystic stage of *E histolytica* as in many instances to be almost indistinguishable.

In the living specimen, the shape is usually oval or spherical, but irregular if motility is present. The size varies from 8 to 15 microns, the average being about 12 microns. Motility is very sluggish, very rarely progressive, and generally consists in the sending out of small, blunt pseudopodia, which are immediately withdrawn, so that no progressive movement results. The cytoplasm appears more refractive than in the vegetative stage, and it is but rarely that any distinction can be made between the ectoplasm and endoplasm, although occasionally the endoplasm appears to be retracted and surrounded by a clear zone representing the ectoplasm. The nucleus is generally visible as a ring-like, refractive body, within which shining granules of chromatin may be observed. At this stage of development the cytoplasm does not contain much extraneous matter and vacuoles are less numerous, and generally entirely absent.

In stained preparations the parasites, in many instances, show but little structure, owing to the intensity with which they take the stain. In well-decolorized preparations, however, the cytoplasm appears granular and more or less reticulated while the nucleus presents a deeply stained, rather thick nuclear membrane, and a distinct karyosome consisting of a deeply staining, spherical mass of chromatin. I have observed in the karyosome evidences of division as shown by thinning of the chromatin and a partial separation of the karyosome into two oval bodies, united by a dimly stained thread of chromatic substance. I have never seen a precystic *E coli* with more than one nucleus, although sometimes it has required considerable care to differentiate cysts containing two nuclei from the precystic forms, in cases in which the cyst-wall was poorly defined. I believe that no reproduction occurs during this stage of development, and that the differences in appearance between the vegetative and precystic forms are, in the latter, entirely preparatory to encystment.

Morphology in the Cystic Stage.—The cysts of *E coli* are characterized by their large size, when compared with *E histolytica*, and when fully developed contain eight nuclei, although this number may be exceeded, as many as sixteen nuclei having been observed. The cysts are found most frequently in formed stools, although in patients suffering from diarrhea the cysts may sometimes be observed along with vegetative forms. This occurs only where the cycle of development has already reached the cystic stage before the stools became semifluid or fluid.

The living cysts of *E coli* are spherical or oval in shape and refractive in appearance The size varies considerably, cysts being observed measuring as much as 25 or 30 microns in diameter, and others as little as 10 microns, but the average diameter is about 15 microns

The cyst-wall varies much in appearance with the age of the cyst, being barely distinguishable as a delicate line surrounding the organism at the beginning of encystment, but presenting a double outline when the cyst is fully developed, the membrane being of considerable thickness Just prior to encystment the organism rids itself of any bacteria, crystals or other extraneous matter that it may contain and the cytoplasm assumes a distinctly alveolar appearance As encystment progresses this alveolar structure is gradually replaced by a finely granular one, and in some of the cysts a large vacuole develops, filling nearly two-thirds of the cyst, the cytoplasm being collected into a granular band surrounding the vacuole, and containing one or two nuclei When two nuclei are present they are often situated at opposite sides of the cyst, separated by the vacuole already mentioned

In some of the younger cysts a few shining granules of chromidia may be observed, but these disappear before division of the nucleus begins, and I have never observed any of the large chromidial masses so characteristic of the cysts of *E histolytica*

The cysts may contain from one to as many as sixteen nuclei, the normal number, when the cyst is fully developed, being eight In the living specimen these nuclei appear as ring-like, refractive bodies scattered through the cyst, and, on account of being situated at different levels in the cytoplasm, it is necessary to focus carefully in order to bring them all into view The cysts that contain more than two nuclei very rarely show the large vacuole mentioned, this being most often observed during the primary division of the nucleus

In specimens of *E coli* stained with iron-hematoxylin or chlorid of iron-hematoxylin, the cystic membrane remains unstained, in most instances appearing as a hyaline band, of single or double outline, encircling the cyst The cytoplasm appears alveolar in the young cysts, but in the older ones it generally is finely granular in appearance In the cysts showing but one nucleus a large vacuola is often present and this may be found in cysts showing two distinct nuclei, but in the cysts containing from four to eight nuclei I have never observed the vacuole, so that it is evident that it disappears as nuclear division progresses In the cysts containing the large vacuole, the nucleus, when single, is of large size and presents evidences of division, the karyosome being divided into two oval or spherical portions, connected by strands of chromatin, or entirely divided In these nuclei the membrane is well stained, of considerable thickness, and not infrequently the nuclear chromatin, instead

of being collected into a distinct, dividing karyosome, appears to be divided into threads running from pole to pole of the elongated nucleus In the cysts containing two nuclei and a large vacuole, the nuclear membrane of each nucleus is distinct, and there may be a well-defined karyosome consisting of a deeply stained mass of chromatin, or evidences of consisting of a deeply stained mass of chromatin, or evidences of division may be noted, the nuclear chromatin being arranged in more or less definite strands within the elongated nucleus In the cysts that do not present the large vacuole, the nucleus, when single shows a definite karyosome, a well-marked nuclear membrane, and granules of chromatin scattered through it between the membrane and the karyosome In such cysts the nucleus resembles closely that observed in the vegetative forms In the cysts that contain two nuclei, however, evidence of division of these is often noted, the nuclei resembling those found in the cysts containing the large vacuole

As nuclear division progresses in the cysts, the resulting nuclei become much reduced in size, are generally spherical in shape, and the elongation and arrangement of the chromatin into threads stretching from pole to pole is not so evident In fact, many of these nuclei consist merely of a well-defined nuclear membrane, containing a loose mass of granules near the center, representing the karyosome In many of them a considerable thickening of the nuclear membrane appears at one portion of the periphery, giving a peculiar cup-shaped appearance to the outline of the membrane

In the cysts with more than eight nuclei the nuclei are still more reduced in size and consist simply of a thin nuclear membrane containing scattered chromatin granules, or a few granules situated at the center, representing the karyosome

It is not unusual to observe cysts that are evidently undergoing degeneration, the cystic membrane being poorly defined and the entire cytoplasm of the cyst being filled with deeply stained granules, threads or masses of chromatin, the nucleus having disappeared, or, if present, being distorted in shape, while the nuclear structure is obscured or atypical in character Sometimes these degenerate cysts contain one or more compact, quite large masses of chromatin, resembling the chromidial masses so characteristic of the cyst of *E histolytica,* but the absence of a definite nucleus, and the smaller size, and irregular shape of these masses should serve to distinguish the cysts containing them from those of the latter parasite

Resistance to Physical Conditions and Chemical Agencies—Practically no work has been done on the resistance of *E coli* to physical conditions or to chemicals, but reasoning from analogy, it is probable that this species follows very closely *E histolytica* in this respect

Relation to Disease—*E coli* is frequently observed in the feces in both health and disease, and although it is probably world-wide in its distribution, the percentage of persons infected varies greatly in different localities, being highest in the tropics and subtropics, and lowest in the temperate zones

This species has been often mistaken for the pathogenic *E histolytica*, and for this reason it is important to know the percentage of persons that may be expected to show it in various parts of the world Schaudinn[1] found it present in 50 per cent. of healthy persons in West Prussia, in 20 per cent in Berlin, and in 66 per cent of healthy people examined on the shores of the Adriatic Sea. In 1905, I[32] examined over 200 healthy American soldiers at San Francisco, recruited from all parts of the United States, and found that 65 per cent of them showed *E coli* in their stools after a dose of magnesium sulphate Later, in conjunction with Ashburn,[33] I found 71 per cent of infections with this parasite among 107 soldiers examined at Manila, Philippine Islands Vedder[45] found 50 per cent of healthy American soldiers infected in the Philippines, and 75 per cent of the native troops Vedder's observations were made on 50 American soldiers and the same number of Philippine scouts Sistrunk[35] found *E coli* in eleven out of 145 patients examined at St Mary's Hospital, Rochester, Minn, the patients suffering from diseases other than dysentery, while Rosenberger,[36] at the Philadelphia General Hospital, found 105 out of 106 insane patients examined infected with this parasite In 1905, I[37] examined the stools of 250 patients suffering from diseases other than dysentery, at the U S Army General Hospital in San Francisco, and found that 49 per cent of them showed *E coli* in their feces

From these data it is evident how widespread is infection with this parasite, and in what a large proportion of persons in both health and disease it may be demonstrated It is also evident that if one were to rely only on the presence of an entameba in the feces in the diagnosis of dysentery, without reference to the species present, every one of these

32 Craig, Charles F Observations Upon Amebas Infecting the Human Intestine, Am Med, 1905, ix, 854, 897 and 937

33 Ashburn, P M, and Craig, C F The Work of the Army Board for the Study of Tropical Diseases in the Philippine Islands, Mil Surg, 1907, xxi, 38, 140, 220, 336 and 527

34 Vedder, E B An Examination of the Stools of One Hundred Healthy Individuals with Special Reference to the Presence of Entamoeba Coli, Jour Am Med Assn, 1906, xlvi, 870

35 Sistrunk, W E Intestinal Parasites Found in Individuals Residing in the Northwest, Jour Am Med Assn, 1911, lvii, 1507

36 Rosenberger, R C Proc Path Soc, Philadelphia, 1912, v, 27

37 Craig, Charles F Observation Upon Amebas Infecting the Human Intestine, Am Med, 1905, ix (reference 103), also The Parasitic Amoebae of Man, Philadelphia, 1911

persons would have been diagnosed as suffering from a latent entamebic dysentery, and this was exactly what was done prior to the work of Schaudinn and his followers

It is also evident that the question of the pathogenicity of *E coli* becomes a most important one, and it has been absolutely proved that this species of entameba is harmless While much work was accomplished prior to Schaudinn's researches, and there was a well-grounded belief that certain species of entamebas were non-pathogenic, it was not until Schaudinn's paper appeared that there were sufficient data to enable one to differentiate the species of entamebas Schaudinn proved on his own person that *E coli* is a harmless parasite, capable of living in the intestine, but incapable of penetrating the mucous membrane or causing the lesions or symptoms of dysentery

In 1905, while studying entamebas at the Army General Hospital, in San Francisco, I tried repeatedly to produce dysentery in young kittens with *E coli,* both by injecting into the rectum material containing entamebas and by feeding similar material containing both the vegetative and encysted forms, but I was never able to produce the least symptom of dysentery, although my experiments with material containing *E histolytica* were successful My experiments were confirmed by numerous investigators and the recent contribution of Walker and Sellards[21] furnishes the final proof of the harmless nature of this parasite

These investigators fed twenty men with material containing the cysts of *E coli,* of which number seventeen became parasitized at the first feeding None of these men developed dysentery, although Walker and Sellards were successful in producing dysentery in some of the men to whom they fed material containing *E histolytica* The period of incubation varied from one to eleven days in the case of *E coli,* with an average of 4 7 days

This parasite may persist in the intestine of man for a very long period of time, and I have personal knowledge of infections with *E coli* that have lasted for from three to ten years, during which time no symptoms of dysentery have ever developed

Differential Diagnosis—Owing to the large proportion of infection with *E coli* in regions where entamebic dysentery occurs, and the ease with which this species may be confused with the pathogenic *E histolytica,* it is most important that the two species be differentiated This may be best done in the living condition, from a practical point of view, but such a diagnosis should, if possible, be confirmed by preparations wet-fixed and stained by one of the hematoxylin methods, preferably Heidenhain's or Mallory's iron-hematoxylin method

In the vegetative stage of development, in living preparations, *E. coli* may be differentiated from *E histolytica* by its sluggish motility, poor distinction between the ectoplasm and endoplasm, large, clearly visible nucleus, and the absence of red blood-corpuscles within the endoplasm, when the stools contain blood In stained preparations, in this stage of development, the parasite is distinguished from *E histolytica* by the thick nuclear membrane, large granules and clumps of chromatin on the inner side of the nuclear membrane, the large, compact karyosome, and the absence of red blood-corpuscles within the endoplasm In the precystic stage of development, in both living and stained preparations, it is exceedingly difficult and often impossible to differentiate this species from *E histolytica,* but there are almost always vegetative or cystic forms present on which such a differentiation may be based

In the cystic stage of development, *E coli* may be differentiated from *E histolytica,* in the living condition by the large size of the cyst, its thicker wall, the absence of large chromidial bodies within it, and the presence of eight or more nuclei, only four nuclei being present in the cyst of *E histolytica* The same differential points are of value in the stained preparations

OBSERVATIONS ON CRAIGIA HOMINIS, CRAIG, 1906, EMEND CALKINS, 1912

In 1906, I observed this organism in the feces of a Filipino suffering from chronic diarrhea, and the same parasite was afterwards observed in the feces of five other Filipinos I published my observations in the same year,[38] giving the parasite the name *Paramoeba hominis,* placing it in the genus *Paramoeba* founded by Schaudinn to include a marine ameba, because of its general resemblance to *P eilhardi,* the type-species of the genus Doflein,[39] suggested that this parasite should be placed in a new genus, as he did not consider that it was a true parameba and, in 1912, Calkins[9] established for it the genus *Craigia*

Regarding the parasite Calkins says

> Craig, 1906, observed a small amoeba in the human intestine in six cases of chronic diarrhoea He named it *Paramoeba hominis* because of the presence of an accessory body which he compared with the *Nebenkorper* of *Paramoeba eilhardi* and the formation of flagellated swarmers The identity of the accessory body with the Nebenkorper is questionable while the single flagellum of the swarmers is sufficient to throw it out of the genus named by Schaudinn For these reasons I propose to give a new generic name —*Craigia*—after its discoverer

38 Craig Charles F A New Intestinal Parasite of Man, Am Jour Med Sc, n s, 1906, cxxxii, 214, also "The Parasitic Amoebae of Man," Philadelphia, 1911, p 215

39 Doflein, F Lehrbuch der Protozoenkunde, Ed 3, 1911, p 603

Further study of this parasite has convinced me that Professor Calkins' criticisms regarding the generic position are just, and I am in agreement with him that it should be placed in a new genus

But very little is known regarding the distribution of this parasite At the time that I described it I had not encountered any infections except in native Filipinos, but in 1908, at Fort Leavenworth, I observed three infections with the organism in American soldiers who had recently returned from the Philippine Islands During the past four years I have been so situated that I have had no opportunity of continuing observations on the parasite, but recently I have been informed by Dr W D Bretz that he has observed a case of infection at Memphis, Tenn, so that it is evident that the parasite also occurs in the United States Indeed, I have always been convinced that a careful study of the feces in cases of chronic diarrhea would demonstrate that *C hominis* has a wide distribution and is a comparatively common parasite, being mistaken in its ameboid stage of development for *E coli,* and in its flagellate stage for trichomonads or cercomonads

Morphology—*C hominis* passes through both an amebic and a flagellate stage of development, and it is quite easy to follow the entire life cycle by making frequent examinations of the feces from day to day, while the symptoms of diarrhea are present There are still certain points regarding the various phases of the cycle that are undiscovered or undecided, but I have been able to study each stage of development including encystment, the formation of the swarmers, the process of longitudinal division of the flagellates, and the development of the amebic from the flagellate stage During each of these phases of development the morphology of the parasite varies so that it is necessary to consider each separately in our description

The life cycle of the organism may be briefly described as follows Beginning with the amebic stage, the parasite reproduces by simple division for a number of generations (as long as conditions are favorable) as a typical ameba, at the end of which time it encysts, and within the cyst there develop numerous small swarmers, which finally escape, develop each a single flagellum, and reproduce by longitudinal division for several generations, after which the flagellum disappears, the organism develops ameboid motion, and again begins the amebic stage of development

For convenience of description the morphology in the amebic stage, the cystic stage and the flagellate stage of development will be considered separately

Morphology in Amebic Stage—The living parasite varies considerably in size in this stage of development, organisms measuring as little as 10 microns being sometimes observed, while others measure as much

as 25 microns in diameter, but the average measurement in diameter at this stage is about 18 microns The resting amebas are round or oval, but when moving they are very irregular, and several pseudopodia may be projected from the periphery at the same time Motility in the fully developed amebas is very active, progressive movement is well marked, and there is a sharp distinction between the ectoplasm and endoplasm, the latter being much more refractive, the ectoplasm being thin and veil-like in appearance In the motionless organism there is no distinction between the ectoplasm and endoplasm, and this is also true of the smaller parasites, in which the motility is very sluggish In the living organisms a nucleus can nearly always be distinguished appearing as a refractive, spherical body, surrounded by a thick and more refractive nuclear membrane of granular consistence Often this membrane appears to be composed of refractile rods arranged end to end around the periphery of the nuclear substance In some of the fully grown amebic forms a brightly glistening oval body, minute in size, may be observed lying near or in contact with the nucleus, appearing much like the blepharoplast of a trypanosome or of the flagellate stage of a Leishmania This is the body that, in my original description, I considered corresponded to the *Nebenkorper* of *Paramoeba eilhardi*, as described by Schaudinn

The ectoplasm is always to be distinguished from the endoplasm in the larger organisms when the parasites are in motion, but when motility is very sluggish in the smallest parasites the distinction is lost, although even in the smallest examples of *C hominis* the ectoplasm is well defined if motility is at all active The ectoplasm appears homogeneous, while the endoplasm is reticular in structure, often giving the appearance of being filled with vacuoles, and it may contain bacteria, crystals and red blood-corpuscles I have not observed vacuoles in the cytoplasm of this species but have no doubt that under favorable conditions they do occur

In stained preparations the ectoplasm and endoplasm are not differentiated, the cytoplasm appearing reticulated and containing bacteria, crystals, and red blood-cells in many instances The nucleus stains intensely, the nuclear membrane being thick and well defined, while the karyosome is large, and separated from the nuclear membrane by an unstained area devoid of chromatin The karyosome stains uniformly throughout, a centriole being absent The nuclear morphology is very similar to that of the genus *Vahlkampfia*, the only difference so far as I have been able to observe, being that this species has a smaller karyosome

The accessory nuclear body is found only in some of the larger organisms It appears as a deep black dot or thick, oval rod, lying near or almost in contact with the nucleus

Organisms undergoing division are observed in stained preparations, and it is apparent the division first occurs in the accessory body, for this body may be seen divided before complete division of the nucleus has occurred During division the chromatin of the nucleus becomes arranged in strands running from pole to pole of the elongated nucleus, and in some instances traces of an equatorial plate may be observed Often the chromatin is collected into two cup-shaped masses at the poles of the nucleus and the space between is filled with fine granules without any definite arrangement Organisms are observed containing two distinct nuclei, each possessing the structure found in the nucleus of adult *Craigia* before division I have never observed any evidences of schizogony in this species

Morphology in the Cystic Stage.—When alive the cysts of *C hominis* are slightly smaller than the adult vegetative forms, measuring, on the average, about 15 microns in diameter, but there is a well-marked precystic stage, in which ameboid motility is lost, the organisms contract and expel any extraneous matter that they may contain, and then begin to rotate rapidly without changing position in the material containing them This process of rotation may last for as long as one hour, but generally ceases in about fifteen minutes, when it will be noted that a distinct cyst-wall has been formed, possessing a double outline, while the nucleus and accessory body may still be differentiated within the cytoplasm As development progresses the nucleus and accessory body disappear and the cyst becomes filled with refractive, spherical granules, which eventually become the young flagellates When fully developed, the cysts of *Craigia hominis* appear as refractive, spherical bodies, surrounded by a well-defined cyst-wall, and containing numerous very refractive spherical bodies

The cysts stain so intensely, evidently because of the richness of chromatin, that it is impossible to make out much in the way of morphology in such specimens In specimens that have been decolorized for a long time one may sometimes see a collection of small ring-like bodies, evidently representing the nuclei of the swarmers, but no fine morphological details can be distinguished

Morphology in the Flagellate Stage.—I have never observed the actual liberation of the flagellates from the cyst, but groups of these organisms are observed arranged in a spherical mass, and surrounded by granular detritus, evidently the remains of the cyst

The young flagellates appear at first as spherical bodies, measuring from 3 to 6 microns in diameter, having a finely granular cytoplasm, and a poorly defined refractive nucleus If these young forms be watched a single, very delicate flagellum appears at one portion of the periphery, and as soon as this occurs, the organism moves forward in a

jerky manner, propelled by the flagellum Contrary to my original belief that the flagellum is situated posteriorly, I now believe it is situated anteriorly, for motion is observed in both directions, and in the fully developed flagellate motion is more often observed in the direction of the flagellum

When fully developed the flagellates measure from 10 to 20 microns in diameter, are spherical in shape, slightly elongated at the portion from which springs the flagellum, and composed of a finely granular cytoplasm in which lies a small, but well-defined nucleus In some of the flagellates the accessory nuclear body may be distinguished as a brightly refractive dot or rod near the nucleus The latter is spherical in shape and has a well-defined, delicate nuclear membrane composed of refractile granules

Reproduction in this stage of development occurs by longitudinal division, and organisms are observed in which every stage of this process may be demonstrated I have not been able to ascertain if the accessory nuclear body divides before the nucleus does, but parasites are observed containing two accessory bodies, two nuclei, and showing commencement of division in the flagellum, while others are observed in which the division of the two flagellates is almost complete, including the flagellum

In stained preparations the cytoplasm of the flagellates stains very intensely and it is difficult to make out the structural details of the nucleus The nuclear membrane is thinner than in the amebic stage of existence, and is apparently composed of minute granules, the karyosome is comparatively large and is surrounded by an unstained area as in the vegetative forms. In fact, the nucleus in the flagellate organisms is quite similar to that in the vegetative stage and markedly resembles that of the genus *Vahlkampfia* The accessory nuclear body stains as a black dot or rod The flagellum stains poorly, the most careful focusing is required to demonstrate it in most organisms In the dividing forms two accessory nuclear bodies may sometimes be distinguished, while the nucleus shows evidences of a primitive form of mitosis, the karyosome dividing and the chromatin collecting at the poles of the elongated nucleus

Relation to Disease—There is no evidence that *Craigia hominis* is a pathogenic parasite, beyond the fact that I have found it present only in patients suffering from diarrhea, and that treatment causing it to disappear also resulted in the cure of the disease I have been unable to produce infection in kittens with material containing this parasite, and there are no reports of pathologic lesions caused by it, so that at present, while I am of the opinion that it is capable of causing a form of chronic diarrhea, its pathogenicity is still unproved

Differential Diagnosis.—In my original contribution I called attention to the likelihood of confusing this parasite with other entamebas and with trichomonads and cercomonads, and described in detail the differential points, but despite this, Hartmann[40] has suggested that I mistook certain stages in the development of *E coli* and *Trichomonas intestinalis* for a new species of intestinal entameba. The absurdity of such a statement must be apparent to any one who has studied the organisms mentioned, and it may be stated that only one entirely inexperienced in the study of the protozoa of the intestine could make such a mistake.

C hominis is distinguished from either *E histolytica* or *E coli* by its small size, the character of the nucleus, the presence of an accessory nuclear body, the structure of the cysts, and the production of swarmers within the cysts. From *T intestinalis* it is distinguished in the flagellate stage by its larger size, the absence of an undulating membrane, the single flagellum, and the spherical shape. In the amebic stage it is distinguished from the resting forms of *T intestinalis*, which sometimes possess a very limited degree of ameboid motion, by the larger size and the active progressive motion, which is never observed in trichomonads.

The differential points mentioned also serve to distinguish *C hominis* from any entameba or intestinal flagellate hitherto described.

It is probable that careful observation will demonstrate that many of the instances of chronic diarrhea now accredited to *T intestinalis* are really due to this parasite, but this will not be determined until there is a more general resort to the microscopical examination of the feces in clinical diagnosis.

OBSERVATIONS ON VAHLKAMPFIA LOBOSPINOSA, CRAIG, 1912, EMEND 1913

The free-living amebas belonging to the genus *Vahlkampfia* are of great interest because of their frequent occurrence in material from cases of entamebic dysentery and the fact that they can be easily cultivated on various culture mediums. The genus also contains parasitic species found in some of the lower animals, and Wenyon,[41] Chatton,[6] James[42] and others have observed vegetative forms of *Vahlkampfia* in feces from the human intestine, so that it appears to be proved that some species of this genus are able to live a parasitic existence in man, but in every instance these organisms were easily distinguished

40 Hartmann, M. Article on "Die Dysenterie Amoeben," in von Prowazek's Handbuch d. Path. Protozoen, 1912, 1, 51.

41 Wenyon, C. M. Intestinal Amebiasis, Brit. Med. Jour., 1913, 2495, p. 1244.

42 James, W. M. Personal communication.

from *E histolytica* and *E coli* and presented the typical limax structure ordinarily observed in cultures

As these amebas occur so frequently in cultures made from feces or material from liver abscesses in cases of entamebic dysentery, almost always as contaminations, the morphology of a typical example of the genus will be described This organism, which I named *Amoeba lobospinosa* in 1912,[43] but which really belongs to the genus *Vahlkampfia*—and for which I now suggest the name *Vahlkampfia lobospinosa*—has a most interesting history It was originally cultivated from the feces of a patient with entamebic dysentery by Dr Musgrave, in Manila, who described it as Ameba 11524 of his series The culture with which I have worked is descended from a culture he sent to the Army Medical Museum in 1905 I have also worked, so far as morphology and life history is concerned, with two other cultures of the same organism, which were sent to the museum by the Board for the Study of Tropical Diseases, in Manila, in 1908 This organism has thus been cultivated on Musgrave and Clegg's agar-medium for a period of nearly eight years, and during this time the morphology has not changed, but has remained that of a typical *Vahlkampfia* In a recent contribution Calkins and Williams[5] have shown that on certain mediums this organism changes considerably in morphology and closely resembles, as they claim, *E histolytica* or *E coli,* and their work will be referred to later

On the medium of Musgrave and Clegg, *Vahlkampfia lobospinosa* presents two well-defined stages of development, the vegetative and the cystic, and the morphology of the organism varies in each stage

Morphology in Vegetative Stage—The size of the organism varies exceedingly, measurements of from 8 to 25 microns in diameter being frequently observed, but the average diameter is about 16 microns The shape, when motionless, is oval or spherical, but when moving the organisms are very irregular in shape Motility is sluggish and progressive motion not very marked, the pseudopodia are of two varieties—short and blunt, and longer and spinose The lobose pseudopodia are present when the organisms are actively motile, but the spinose pseudopodia are more frequently observed when motility is absent or very sluggish The spinose pseudopodia are extruded from the periphery of the ameba very rapidly and as rapidly withdrawn, so that it is often difficult to distinguish them Both lobose and spinose pseudopodia may be present at the same time

The nucleus is well defined, appearing in the living organism as a spherical, refractive mass surrounded by a narrow hyaline area, while the nuclear membrane is indistinct In most of the vegetative forms

43 Craig, Charles F Observations Upon the Morphology of Parasitic and Cultural Amoebae, Jour Med Research, 1912, xxi, 1

a single, contractile vacuole is present, which contracts once in about fifteen seconds, and which, when most clearly defined, is situated near the nucleus but travels toward the periphery before it contracts In most of the organisms the contractile vacuole appears to be situated at the periphery when contraction occurs

In stained specimens the distinction between the ectoplasm and endoplasm, which is quite marked in the living condition, is lost, and the entire cytoplasm stains a brownish gray or gray, appears reticular in structure, and composed of granular material The nucleus is characteristic in structure, being composed of a large karyosome, filling at least two-thirds of the entire nucleus, surrounded by a clear unstained area, which, in turn, is surrounded by a zone of fine chromatin granules forming a poorly stained nuclear membrane The karyosome stains intensely and, unless the preparations are well differentiated, does not show a centriole, but in well-stained and decolorized preparations a centriole may be observed within the karyosome, consisting of one or two almost black dots The unstained area surrounding the karyosome is devoid of chromatin granules and no traces of a linin network can be distinguished in this area In many of the vegetative forms the nuclear membrane cannot be distinguished from the cytoplasm, but it may appear well stained and then consists of a rather thick membrane, on the inner side of which there is a zone of fine chromatin granules

The cytoplasm contains many bacteria and often the single vacuole is well differentiated, together with unstained areas demonstrating the reticular nature of the protoplasm

During the vegetative stage of development reproduction occurs by simple division, the nucleus often showing well-defined mitotic figures Organisms are observed in which the karyosome as well as the entire nucleus is greatly lengthened, while evidences of division of the centriole are noted in well-differentiated preparations A later stage in division is shown in organisms having deeply stained crescentic bodies at each pole of the nucleus, connected by fine filaments of poorly stained material, the whole forming a well-defined spindle An equatorial plate is not infrequently observed consisting of a band of deeply stained granules of chromatin stretching across the spindle midway between the polar bodies, and sometimes a distinct division of this plate into two rows of deeply stained granules may be observed Still later the equatorial plate and the spindle disappear and the nucleus is divided into two portions, spherical in shape, each having a distinct karyosome and a poorly defined nuclear membrane In this species the mitotic character of the division of the nucleus is well marked, much more so than it is in the intestinal entamebas

Morphology in the Cystic Stage—*Vahlkampfia lobospinosa* produces cysts whenever the cultural conditions are unfavorable to vegetative existence, and on the ordinary culture mediums the cysts are characteristic of this genus, only one daughter ameba being formed in each cyst In size the cysts vary from 8 to 15 microns in diameter, the average being about 10 microns The youngest cysts, when living, have a delicate cyst wall, of single outline, but the fully developed cysts show a thick wall, very refractive in character, and having a well-marked double outline The cysts are spherical in shape so long as the medium is moist, but as drying occurs they contract unequally and irregular forms are produced, generally quadrilateral or pyramidal in shape The old cysts often have a very thick wall which appears cracked or folded over in places, thus giving the cyst a very characteristic appearance The cytoplasm of the young cysts appears finely granular but free from bacteria, and the single nucleus may be observed near the center of the cyst In the older, living cysts the nucleus is often invisible and the cyst appears to be filled with minute refractive granules the nature of which I have been unable to determine

In stained preparations the cytoplasm appears granular and the nucleus presents the same structure that is observed in the vegetative forms before division occurs I have never observed more than one nucleus in a cyst, nor have I ever observed any evidences of division of this nucleus, so that encystment in this species, so far as it is shown on ordinary culture mediums, is purely a protective process, as reproduction does not occur within the cyst

In brief, the description given above answers to the appearance of *V lobospinosa* as observed in cultures on Musgrave and Clegg's nutrient agar, but the recent work of Williams and Calkins[6] has shown that when this species is grown in pure culture on brain-tissue medium very marked variation occurs in its morphology from what occurs when it is grown, in conjunction with bacteria, on ordinary mediums They have found that the contractile vacuole disappears from the vegetative forms, the nuclei resemble in structure the nuclei of the entamebas, and the cysts become multinucleate, from two to four nuclei being present in many of them Williams and Calkins consider these variations as significant of the parasitic nature of the species, but are very conservative regarding their exact significance, saying [6] "We do not wish, however, to lay too much stress upon these experimental results, conditions of the culture might be such as to bring about variations when these do not occur under conditions 'normal' to the organism" These observations are of the utmost interest as showing how profoundly a protozoan organism may be modified by unnatural cultural conditions, but there is no reason to believe that these modifications in any way

connect this typical free-living ameba with the entameba of the intestine, as *E histolytica* or *E coli*, for even though the structure of the nucleus is modified it is perfectly evident to anyone who has worked with the entamebas that none of the photomicrographs published with the paper of Williams and Calkins show amebas with a nucleus in the least like the nucleus of either *histolytica* or *coli*, while even in the drawn plate the nuclei are markedly dissimilar in most of the drawings It is a fact that whenever a *Vahlkampfia* has been found parasitic in the human intestine, and this has occurred in a few cases, as mentioned, the morphology has been typical of the limax type of ameba and the forms observed by these authors have never been noted As regards the relation of these free-living species to disease the observations of Walker and Sellards, hereafter considered, are conclusive

Relation to Disease.—There is at present no evidence that any species belonging to the genus *Vahlkampfia* is capable of producing disease in man, and it is evident from the investigations of many authorities that, in most instances, the species of this genus are actually incapable of establishing themselves as parasites in man They are all essentially free-living amebas, so far as their relation to man is concerned, and while a very few instances have been reported of infection with the vegetative forms of *Vahlkampfia*, the infection lasted but a short time and no symptoms or lesions were produced by the organism Therefore, we are justified in believing that such instances are accidental, and while they may be interesting from the point of view of the evolution of parasitism in a species, at present they possess no practical importance to the clinician

To consider all of the observations that go to prove the fact that the species of the genus *Vahlkampfia* are essentially free-living as regards their relation to man would be only to repeat, but the recent work of Walker and Sellards, in my opinion, proves conclusively that the ordinary cultural amebas bear no relation whatever to the intestinal entamebas, but are in nature free-living and incapable of existing as parasites in man

Walker and Sellards[21] experimented on ten men by feeding them cultures representing thirteen different strains and eight species of free-living amebas isolated from the Manila water-supply and other non-parasitic sources, from the stools of healthy persons and persons suffering from diseases other than dysentery, and also from dysenteric stools They found that when cultures of these amebas were ingested by the men the amebas could be recovered from their stools in culture during the first few days after feeding but not subsequently, and that the microscopical examination of such stools always resulted negatively No symptoms of dysentery occurred in any of the men, although they

were observed for months, and as the result of their work the authors draw the following conclusions

1 The cultivable amoebae are incapable of living parasitically in the intestinal tract of man

2 The amoebae, when obtained in cultures from stools, intestinal contents, or liver-abscess pus, are derived either from cultural contaminations or from encysted amoebae which have been ingested with water or food and have passed unchanged through the intestinal tract

3 The cultivable amoebae are non-pathogenic, and consequently play no rôle in the etiology of endemic tropical dysentery ,

In the light of these conclusive experiments it is very evident that no amount of evidence based on mere changes in morphology or life cycle produced by growing free-living amebas on unnatural culture material can be considered as of the slightest value in connecting them with the true entamebas, but that at most these variations simply indicate the path by which the present species of entamebas reached their parasitic mode of existence From the point of view of evolution the experiments of Williams and Calkins are most valuable and interesting, but they in no wise affect the present classification of the parasitic species I believe that it is proved that the cultivable amebas cannot, in the vast majority of instances and under natural conditions, live as parasites in man, and that they are entirely distinct from the entamebas and bear no relation to the etiology of entamebic dysentery

In conclusion I desire to state that the personal observations recorded in this contribution were made at the U S Army General Hospital, San Francisco, Cal , the Division Hospital, Manila, P I , while the writer was a member of the U S Army Board for the Study of Tropical Diseases in the Philippines, the Rockefeller Institute for Medical Research, New York, the Laboratory of the Army Medical School, Washington, D C , and the Department Laboratory, Central Department, U S Army, at Fort Leavenworth, Kansas For the photomicrographs, of which Figs 1 and 2 are reproductions, I am indebted to Capt Arthur C Christy, U S Army They were made from preparations stained with iron hematoxylin and fixed with Schaudinn's sublimate alcohol solution

THE PHYSIOLOGIC DEGENERATION OF THE MESONEPHROS

WITH A CONSIDERATION OF ANTAGONISTIC ACTION OF ORGANS AND OF FUNCTIONAL MUTATION WITH MORPHOLOGICAL PERSISTENCE [*]

HORST OERTEL
LONDON, ENGLAND

I propose to draw attention to certain embryological processes and phenomena which appear to have escaped a more than cursory attention by the pathologist and, if a perusal of the literature and an inquiry among those able to judge lead me to suppose correctly, have received a rather inadequate consideration by anatomists and physiologists The road, therefore, is difficult to travel, and the observations and considerations which are recorded below will appear necessarily fragmentary and incomplete Indeed, the difficulty of obtaining suitable material and other more personal obstacles allow me to offer no more than an outline of the matter That, however, seems warranted by the importance and the unfortunate neglect of the subject Even in incomplete form it may, nevertheless, obtain the interest and future help of those who by position and experience are able to enlarge on the facts and elaborate the conclusions to be drawn therefrom

Perhaps it may not be amiss to review briefly, as an introduction, our present knowledge and ideas regarding renal development in general and the mesonephros in particular

I INTRODUCTORY GENERAL PART[1]

It is of course impossible to present within the scope of this paper, which proposes to deal only with certain phases of nephric development, a thorough discussion of the whole complex and still disputable subject of the origin of the excretory system and the relations it may have to the original annelid nephric organ These to the pathologist by no means unimportant questions, must be studied elsewhere [2] I shall con-

* From the Gordon Pathological Laboratory of Guy's Hospital
* Submitted for publication March 17, 1914

1 The introductory general part was essentially written for those unacquainted with present views of renal development and of the position and structure of the mesonephros, others may turn immediately to the second special part

2 Consult Felix, W The Development of the Urogenital Oragns, in Man-

fine myself to a sketch of those features a knowledge of which appears necessary for an intelligent understanding of the subject at issue

The kidney of all amniotes discloses, as is well known, its phylogenetic travels much plainer than any other organ By succession and partly coexistence appear three distinct excretory organs, pronephros, mesonephros and metanephros The pronephros disappears rapidly, but the mesonephros, or wolffian body, acquires full development and a high degree of differentiation In man, particularly, this rises to a better state of evolution than in some of the lower animals, in pigs it reaches an enormous size and recedes only in embryos of 10 cm , while in reptiles it continues through part of the postnatal life Thus in lizards the mesonephros persists to the end of the first year and, according to Braun,[3] retrogression of the mesonephros does not commence until after the first hibernation On the other hand, it happens that in the mouse the wolffian body develops only incompletely and is even devoid of all glomeruli

It is, therefore, evident from the start that the development of the amniotic kidney is quite unlike the gradual, uniform development of other organs, but *serial,* for it appears that pronephros, mesonephros and metanephros are to a considerable extent at least independent organs, which take origin from closely adjoining and partly overlapping segments of the mesoderm, so that the pronephros develops most cranially, caudally to it the mesonephros and still more caudally the metanephros or permanent kidney. The exact genetic relation of these three organs has been, and still partly is, a disputed question For our discussion it is sufficient to emphasize that a relation exists so far as they represent renal excretory organs patterned on one structural scheme, but of a growing complexity which is demanded by progressively higher grades of general development, and corresponds to various stages of animal evolution As one is replaced by the other the predecessor appears thereafter by recapitulation, rudimentary or provisional A certain

ual of Embryology, edited by F Keibel and F P Mall, Philadelphia and London, J B Lippincott Company, 1912

Felix, W Die Entwicklung des Harnapparats (1904), in Hertwig's Handbuch der vergleichenden Entwicklungslehre der Wirbelthiere, Jena, G Fischer, 1906

Gaskell, W H The Origin of Vertebrates, London, etc , Longmans, Green & Co , 1908

Broman, Ivar Normale und abnormale Entwicklung des Menschen, Wiesbaden, Bergmann, 1911

Bonnet, R Lehrbuch der Entwicklungsgeschichte, Berlin, Paul Parey, 1907

3 Braun Das Urogenitalsystem der einheimischen Reptilien, Arbeiten aus dem zool -zoot Institut zu Würzburg, 1877, IV

Schreiner, K E Ueber die Entwicklung der Amnioten Niere, Ztschr f wissensch Zool , 1902, lxxi, 1-189

structural resemblance may, therefore, be noted in all three which in amniotes is necessarily closest between mesonephros and metanephros In myxinoids the pronephros represents the permanent kidney In fishes and amphibia it atrophies and is replaced by the better-organized mesonephros Finally, in amniotes the mesonephros also undergoes retrogression and is replaced by the most highly organized metanephros as permanent kidney One other and more special relation between these three organs must be noted There is developed in connection with the pronephros an excretory duct, called the primary excretory or segmental duct, which takes origin from the thirteenth and fourteenth and possibly other caudal segments of the pronephric anlage It penetrates early into the cloaca From it descend directly the wolffian duct of the mesonephros and the ureter of the metanephros with their derivatives

An understanding of the functional and structural characteristics of all three kidneys is much helped by tracing them through phylogenetic evolution In the lowest unicellular forms of animal life the general surface of the cell serves as a medium for the discharge of waste products But under certain conditions, probably increased energy, there appear contracting vacuoles in addition to others of alimentation and distribution The contracting vacuoles have been regarded as the first evidences of an excretory system Thus Rhumbler believes that certain excretory granules and crystalline enclosures which were first observed by Stein in 1859, and later thoroughly investigated by Butschli, are discharged by pulsating vacuoles The nature of these granules or crystals is held to be either uric acid (Griffiths, 1889) or an oxalate (Butschli) or calcium phosphate (Schewiakoff) [4]

We see further in the lower metazoa, such as the turbellarins, a complex system of tubes which ramify in the tissues of the body and constitute an independent excretory organ

The appearance of the coelom or body cavity marks the beginning of a definitely recognizable excretory system which was combined in all probability from the start, or at least very early, with the sexual apparatus This combination persists to the human organism, in which a separation of urinary and sexual organs has only partly been accomplished Both form a unit during human embryonic and fetal life, and even in the adult one channel serves both as terminal passage At the earliest stages of evolution both were represented by the coelom walls which possessed the ability not only of elimination, but also of producing sexual cells, gonads (Gegenbaur[5]) Gradually the coelom developed ducts

4 Von Furth, O Vergleichende chemische Physiologie der niederen Thiere, Jena, G Fischer, 1903

5 Gegenbaur, C Vergleichende Anatomie der Wirbelthiere, Leipsic, W Engelmann, 1898

for conveyance of the excretory products to the exterior It is very probable that later a certain portion of the coelom wall became reserved for the sexual function and another for the excretory function

Thus originated sexual glands and pronephros, the first definite excretory organ of vertebrates Notwithstanding their appearance, the association of the two systems continues quite intimate throughout the whole series of vertebrates, and is necessarily most marked during early life

In this persistent and peculiarly characteristic interchanging relationship of excretory and sexual apparatus rest, as will appear, important factors which seem to enter into the developmental fate of both

It has been stated that the structural organization of the three excretory glands is based on one scheme This is dictated by their two main functions These are (if we neglect for the time certain metabolic activities which acquire importance only in higher animals)

1 To remove useless or dangerous metabolic or foreign substances from the body (excretion)

2 To allow permeation and elimination of superfluous water from the body fluids (filtration)

Both combined maintain constancy in the composition of the body-fluids and the blood It is very probable that the most primitive urinary organ, the coelom, or body cavity, was able to carry on both functions Each coelom cell possessed excretory activity, and the coelom wall as a whole formed a membrane permeable for water With growing complexity of differentiation it became necessary to specialize these functions in particular parts of the coelom cavity Consequently that portion of the coelom wall which was richly provided with blood-vessels assumed filtrative function by the formation of the external glomerulus This consisted only of an invagination of blood-vessels into the body cavity On the other hand, the transverse ducts remained reserved for the more specific excretory function This constituted the pronephric period During later stages of development the filtration apparatus of the external glomerulus, originally quite independent, was incorporated in the kidney and quantitatively modified to adapt itself to the new environment and demands Thus originated the malpighian corpuscles of the mesonephros and later of the metanephros This union created an organ which again combined, as did the coelom, filtrative with excretory functions, but in a much higher degree of efficiency At this point all communication between kidney tubules and coelom disappeared, and the latter retained only the mechanical function of separating movable organs from each other and the body walls

The fact that the kidney of higher animals represents a union of originally independent units is of some interest in relation to its func-

tional activities, for it is likely that both units have largely retained their chief original purposes, the one of filtration, the other of secretion and excretion In this connection it is interesting to recall that with progressing evolution the tubular secretory structures become not only more numerous but also more complex, and that their cells undergo various processes of differentiation They show, therefore, important qualitative tissue changes It is different with the glomerulus From the one external glomerulus a step to many is made in the mesonephros and a further step to a still greater number in the metanephros But the glomerular structure, unlike the tubules, remains essentially the same throughout evolution One would conclude that glomerular function, although quantitatively enormously increased, has not undergone marked qualitative changes

To recapitulate and sum up All three kidneys are evolutionary stages of an organ series They have in common secretory tubules opening into an excretory duct, but their structures arise from different segmental portions of the mesodermal plate, and, therefore, are not identical or evolutionary periods of one organ At first, during the period of tubular formation from the cranial segments, the tubules are simple and retain segmental arrangement A glomerulus lies outside and independent to the secretory portion (pronephros) Later, when formed in the dorsal and lumbar regions, tubules appear in greater number and become more complex, and glomeruli appear and are incorporated and connected to the tubules in a fully developed renal organ (mesonephros) In the last stage of sacral development the tubules assume greater complexity and number with a corresponding increase of glomeruli, and they first group themselves around and then unite with an outgrowth from the wolffian duct the ureter and its derivatives, all of which are the direct descendants of the pronephric primary excretory duct (metanephros)

This short, rather crude sketch may perhaps suffice to recall to mind the position which the mesonephros occupies in genetic and individual history, and we may now briefly review its structure and organization

Attention has been drawn to the fact that the mesonephric tubules develop from those stalks of the primitive mesodermal segments which lie caudally from the pronephros In mammals these form early a blastema or cell cord which gives rise in its cranial portion to the parenchyma of the mesonephros and later in its caudal portion to the parenchyma of the metanephros This blastema is commonly spoken of as the nephrogenic cord, and its appearance terminates the existence of the critical period of individual segment stalks and inaugurates the possibility of a greater number of tubules of growing complexity These tubules empty into the primary excretory duct, which now becomes the wolffian duct

Recent investigations have settled the long dispute about the manner of kidney development in favor of a dualistic origin, according to which one anlage gives rise to the secretory, and another to the excretory part The mesonephros, therefore, like the metanephros, later is formed by a union of parenchyma, that is, glomeruli and secretory tubules, derived from the nephrogenic cord with an outgrowth from the wolffian duct, that is, the collecting tubules In the metanephros the parenchyma is joined to the pelvis of the kidney by the collecting tubules, both of the latter representing an outgrowth of the original ureter and termed the ureteric tree

In appearance, structure and organization the mesonephros, therefore, bears such a striking resemblance to the permanent kidney that it may justly be regarded as an abbreviated metanephros

Depending on its mesodermal derivation, the mature mesonephros is situated anteriorly to the position occupied later by the metanephros, that is, the lower dorsal and upper lumbar or hepatic regions In relation to the embryo it is large and presents in shape a broad, slightly curved mound or ridge Its malpighian corpuscles are similar to those of the permanent kidney and show well-developed glomeruli filled with blood and occupying the whole of the capsule The latter is plainly lined by rather high epithelium Marked differences exist between the convoluted and collecting tubules The convoluted tubules bear, except for number, a striking resemblance to those of the metanephros, more particularly in regard to their lining epithelium, which present the general characters of all renal secretory epithelium in convoluted tubules Mihálkovics[6] describes these cells as high, cylindrical (from 0 01 to 0 02 mm), of yellowish, poorly staining protoplasm and distinctly granular But Winiwarter and Sainmont[7] speak of them as cuboid epithelium, which I think is more correct for the intact undegenerated cells Individually, the cells are sometimes poorly outlined so that, according to Mihálkovics, this epithelium appears in sections frequently like a nucleated yellowish syncytium The nuclei are relatively large, round and rich in chromatin, and possess, according to Winiwarter and Sainmont, a large nucleolus In early stages they show frequent mitoses Mihálkovics describes irregular, colorless, thread-like processes (cilia) extending from the free cell ends into the lumen of the tubules, in which they seem to be continuous with a network which fills the wide lumen He interprets these pictures as evidences of

6 Mihálkovics, G Von Untersuchungen uber die Entwicklung des Harn und Geschlechtsapparats der Amnioten, Internat Monatsschr f Anat u Histol, 1885, II, p 41 and p 65 , ,

7 Winiwarter, H Von, and Sainmont, E Nouvelles recherches sur l'ovogénèse et l'organogénèse de l'ovaire des mammifères (chat), Arch de biol, 1909, XXIV, 373 Involution du corps de Wolff

glandular secretion Winiwarter and Sainmont also observed the discharge of hyaline globules from these cells which they regard as secretion I have been unable in the material at my command to discover similar structures except in degenerative processes As, however, fixation and freshness as well as hardening and staining methods materially influence such details, I do not feel able to deny their existence Whether or not they really represent products of a secretory activity is, of course, open to question

In the straight and narrow collecting tubules which can be distinguished as easily from the convoluted ones as in the metanephros, the lining epithelium is low (from 0 007 to 0 008 mm , Mihálkovics), closely set, very distinct, clear, with smaller nuclei and resembles the lining cells of the wolffian duct, indicating a close relation of that tubular portion to the excretory duct

The whole mesonephros is enveloped by a capsule composed in the earlier stages of development by several loosely arranged and cellular layers, in the mature organ, however, the capsule shrinks, and its cellular contents are lost The parenchymatous or cortical part shows early many undifferentiated blastemic cells lying in a loose, thin stroma As the tubules develop, these diminish, and in the fully developed mature mesonephros practically no interstitial cellular tissue is to be seen between the tubules of the cortex, but these lie in close proximity, separated only by thin but distinct basement membranes and intertubular capillaries A thin, loose stroma is retained around the malpighian corpuscles This is more abundant toward junction with the wolffian duct

If, then, as will be apparent from the foregoing description, there exists an essential similarity in the organization of the mesonephros and metanephros, there are also, regardless of details, two very important differences, first in circulation, secondly in the connection with the sexual gland These must, therefore, be shortly described

The mesonephros enjoys a very rich and peculiar arrangement of its blood-supply It receives in the first place a large number of arteries from the lateral aortic side According to Bonnet,[2] there are no less than twenty in the fully-developed human mesonephros, but of even greater abundance is its venous portal circulation (Felix) In order to understand this properly one must recall that the mesonephros lies between the vena cardinalis posterior on the dorsal side and the vena subcardinalis on the ventral side Both veins are connected at its caudal border with the venae caudalis and ischiadica Early in mesonephric development numerous branches begin to form between the posterior cardinal and subcardinal veins, traversing the mesonephros From the beginning the vena cardinalis posterior opens into the duct of Cuvier and through this into the sinus venosus of the heart, while the vena subcardinalis acquires

connection with the heart through the hepatic vein (Felix) We shall have reason to refer later to these peculiar circulatory conditions

The second peculiarity of the mesonephros — its intimate connection with the sexual gland — will be referred to here but briefly, for it will form the subject of a later discussion At a variable, but very early stage of mesonephric development, a narrow strip of mesothelium extending along the medial surface becomes prominent and cellular, forming the genital tubercle These cells are later arranged into layers and become definitely differentiated and grouped The whole mesothelial band is known as germinal epithelium and gives rise to the sexual glands, testis or ovary A connecting band remains and is known, respectively, as mesorchium or mesovarium

Lastly, the time over which mesonephric development extends is not uniform in amniotes, even in the mammals, in which it remains a purely embryonic organ Traces of an early original segmental arrangement have been observed up to human embryos, but the segment stalks fuse rapidly to the nephrogenic cord, so that the early differentiation of the mesoderm is, in mammals at least, much abbreviated

The time of appearance of the first mesonephric tubules varies It has been tabulated by Felix, as follows In the rabbit from the ninth to the tenth day (Kollicker, 1879) 245 hours after fertilization in embryos of from 5 to 5 5 mm greatest length, a dog, eighteen days, four hours after coition showed from sixteen to twenty primary segment pairs and from eight to seventeen anlagen (Bonnet, 1887) In the pig the first mesonephric anlage is found fourteen days, nine hours after coition in embryos of from 5 to 5 3 mm greatest length in ten or eleven primary segment pairs; in man the first tubules appear in embryos between 2 6 to 3 mm greatest length (His, 1880; Keibel, 1896) The mesonephros is reported as complete in the guinea-pig on the twenty-third day after the last litter (Weber, 1897), in rabbits between 18 and 20 mm length (Mihálkovics), or on the sixteenth day of development (Egl, 1876), in sheep of from 18 to 20 mm length (Weber, 1897), in pigs of from 6 to 7 cm length (Keibel, 1897, Neuhauser, 1903), in human embryos of 7 mm length (Weber, 1897, MacCallum, 1902), and in the mouse the development, as mentioned, remains incomplete and no glomeruli are formed (Weber, 1897) When the mesonephros has reached its caudal limit, no new caudal anlages are added in older embryos, and a degenerative and destructive process becomes generally manifest which annihilates the organ with the exception of those anterior and middle parts which enter the services of the sexual organs The manner, character and bearing of this process will form the subject of the following special part

II SPECIAL PART

The degeneration and ultimate fate of the mesonephros have been much discussed by anatomists from the developmental point of view, but the histological changes leading to its loss, their causes and possible bearing on certain problems of postnatal pathology have hardly received any attention Nearly thirty years ago (1885) Mihálkovics[6] contributed in a classic work an investigation and description of the histological details Nothing has been added since, with the exception of the recent work on the organogenesis of the cat's ovary (1909) by Winiwarter and Sainmont,[7] who describe the degenerative appearances in the mesonephric tubules with care, and in the two most complete expositions of the subject by Felix, the one in Hertwig's "Handbuch der Entwicklungsgeschichte," the other in Keibel and Mall's "Human Embryology," only a short summary of Mihálkovics' observations are given But this matter is perhaps of greater pathological than embryological interest, for these phenomena resemble very closely the changes in certain metanephric diseases Nevertheless, they occur without bacterial or outside toxic invasion, but as a part of a *normal* developmental process They deserve, therefore, special recognition and study by the pathologist

The degeneration of the mesonephros commences early in mammals In the human embryo, according to Felix, even before the mesonephric development has been caudally completed, in an embryo of 5 3 mm, it involves primarily the most cranial tubules and proceeds rapidly to embryos of 21 mm This constitutes the first and essential part of mesonephric degeneration The second period is said by Felix to commence very gradually, progresses slowly and is characterized by incorporation of the middle mesonephric portion into the genital system All other parts undergo complete retrogression It will be remembered that of the remaining parts in the female (paroophoron, duct of Gartner, parovarium), none retains any visible function, while in the male (vasa efferentia, coni vasculosi, paradidymis, epididymis, vas deferens, ejaculatory duct, seminal vesicles), the mesonephric remnants become new functionating structures

A study of the degeneration of the mesonephros involves necessarily the consideration of three questions

1 What are the histological changes leading to annihilation of the organ?

2 What is their cause?

3 What is their significance?

1 It has already been noted that the most complete study of the histological changes leading to loss of the mesonephros is that of Mihálkovics made in 1885 This description is so excellent and characteristic that I shall quote it in full Mihálkovics studied the lesion in chicks,

commencing with the eighth to ninth day of incubation, and in rabbits of from 16 to 18 mm lengths at these times the formation of mesonephric buds and tubules has practically come to a standstill, the tubules no longer develop convolutions, but remain stationary, while the large venous sinuses recede and assume the dimensions of usual blood-vessels The interstices and recesses thus formed are filled by connective tissue which previously has existed only sparingly and loosely between the tubules At the same time the epithelium of the convoluted tubules gradually loses the character of secretory cells and assumes the shape of ordinary cylindrical cells with a greater affinity for stains than possessed before The tubules persist in this state, in chicks, from the eighth to the sixteenth or seventeenth day, in the rabbit to a length of from 3 5 to 4 cm Then they commence to obliterate The first evidence of this obliteration is a narrowing of the convoluted tubules with a progressing small-drop fatty degeneration of the lining cells Ultimately the cell bodies undergo a fatty dissolution and fill the lumen of the tubules Instead of canals there remain thus solid cell cords which consist of still recognizable tubular cells These are more or less concealed by the richly cellular stroma and may, therefore, escape attention In the meantime the malpighian corpuscles have become involved in the process Their loss commences with obliteration of the afferent vessels, and the atrophic glomerulus no longer fills the capsule, but leaves a space between the tuft and capsule wall Finally, the capsule, following the receding glomerulus, retracts and becomes attached to the tuft In sections these remnants of malpighian corpuscles resemble cell buds or groups lying between obliterating mesonephric tubules One other point was fully noticed by Mihálkovics, although he drew from it no theoretical or hypothetical conclusions, namely, that as the mesonephros shrinks, the medially attached genital gland enlarges, thus reversing the relative size of the two organs "Primarily the small genital gland rested on the medial side of the massive mesonephros, after its contraction insignificant rests of the mesonephros lie on the lateral side of the now huge genital gland" Mihálkovics, however, did not trace further the details of this impressive phenomenon Later investigations have generally recognized and recorded it also without further comment

The recent observations of Winiwarter and Sainmont on the organogenesis of the cat's ovary have supplemented in certain details Mihálkovics' work They found that the transformation of those mesonephric parts which go to form epoophoron and paroophoron commences with a pigmentary degeneration of the lining cells of the convoluted tubules This epithelium becomes swollen, coarsely granular and later desquamated, and in advanced degeneration narrow tubular canals are found whose lumen is filled by pigment-granules It will be observed that the

changes described by Mihálkovics and Winiwarter and Sainmont bear close resemblance to pathological lesions A further study, especially regarding their histogenesis and causes, seemed, therefore, desirable, for such knowledge might prove of value in the interpretation of pathological processes

The material at my disposal was by force of circumstances limited, so that observations could not be made on a desirable extensive and detailed scale Much remains hidden which must be cleared by future investigation But the results are perhaps of sufficient interest to warrant this fragmentary report, more especially as they differ somewhat from previous views [8] The available material consisted of rabbit embryos from the fourteenth to the twenty-sixth day of intra-uterine life, and marsupial embryos of *Dasyurus viverrenus,* beginning with the fifth to sixth day and extending to the nineteenth day To obtain the full age of these embryos, about eight days intra-uterine life must be added

These periods comprise from the early to the most active stages of mesonephric destruction The lesions appeared essentially the same in both animals The results are, therefore, presented together

Certain histological modifications distinguish the destruction of the anterior portion of the mesonephros, which enters the service of the sexual gland from the posterior one, which is completely lost The involvement of both, however, becomes evident immediately after beginning growth of the sex gland and goes hand in hand with the latter's development and differentiation In rabbits this commences from the fourteenth or the fifteenth day, in the dasyurus after about nine days of marsupial life, and then proceeds with great rapidity To begin with the changes in the anterior portion of the mesonephros which lies in closest contact with the sex gland when an enlargement of the sex gland, which until then has only formed a thickening or tubercle on the medial mesonephric side, is noticeable, there occurs in the vicinity of attachment to the mesorchium or mesovarium a small cell proliferation which leads by rapid extension to a marked interstitial cell infiltration of the neighboring parts It thus involves diffusely the periglomerular tissue, but also spreads peripherally under the outer capsule From these points it penetrates by streaks into the glandular parts between the convoluted tubules

The periglomerular infiltration surrounds and compresses the malpighian corpuscles, and involving Bowman's capsule, invades their interior and gradually obliterates it The replacing cells have all the appearances of connective-tissue cells, first round, they soon acquire a distinct small and large spindle shape During this process the glomerular tuft is much

8 I intend to publish at some later day a full, illustrated account of mesonephric degeneration

engorged, and hemorrhagic extravasations into the capsule, sometimes sufficient to destroy the whole tuft, may occur This loss of malpighian corpuscles in the anterior portion appears to be one of the earliest and most rapid manifestations of mesonephric degeneration

Proceeding from this focus, streaks of interstitial cells extend to the territory of the tubules, where they meet similar invading cells from the subcapsular tissue This progress leads to marked thickening of the previously very thin intertubular septa of the convoluted tubules, and is associated with a rather irregular engorgement and dilatation of capillaries

Coincident and probably not, or at least not solely, dependent on this cell infiltration, is marked parenchymatous degeneration of the cells lining the tubules, closely followed by desquamation and proliferation of this epithelium The cells become succulent, swollen, coarsely granular, in many pigmentary granules appear, or small to large vacuoles (fat?) and hyaline drops They are then desquamated as a whole, or discharge granular masses and pigmented granules into the lumen, forming casts The nucleus is well preserved to the last, and seems to succumb by karyolysis

Proliferation of the epithelium is very active, so that frequently several cell layers are formed But the new cells are soon overtaken by a similar fate In tubules in which the cells appear more resistant or are perhaps later attacked, they seem to become higher and narrower, cylindrical, as Mihálkovics terms it, before they also undergo disintegration

Ultimately, the tubules are invaded and compressed by interstitial cells from the septa, broken and obliterated The collecting, straight tubules remain well preserved until late and are apparently lost by cell overgrowth without exhibiting the degenerative changes so conspicuous in the convoluted tubules

Thus the anterior part of the mesonephros is generally overgrown by cellular fibrous tissue and contracts, and the remaining uninvolved tubules of the middle portion are drawn toward the territory of the meanwhile enormously enlarged sex gland, where they may be recognized for some time surrounded by richly cellular connective tissue

Somewhat different seems to be the fate of the posterior portion of the mesonephros which is doomed to complete annihilation Degenerative features seem to predominate in it almost entirely, and sclerotic changes do not take an active part in its destruction

The changes appear very nearly about the same time as anteriorly, possibly somewhat later Toward the end they proceed with great speed

The glomerular coils become cellular and show swelling, turbidity and gradual fusion of capillary loops This may involve at a time only a number of loops, and the remaining ones show then a marked capillary dilatation and engorgement The fused coils become generally adherent

to some part of the capsule, very much as in nephritis, the glomerulus retracts and the capsular space is widened The capsule wall shows usually swelling, but no cellular thickening, and remains intact until the whole capsule collapses from loss of the glomerulus Such pictures resemble those described by Mihálkovics, but I have been unable to observe the swelling and obliteration of the glomerular afferent vessel to which he ascribes glomerular retraction and loss On the other hand, the degenerative changes in the convoluted tubules are similar to those in the anterior portion, and there exists also irregular intertubular engorgement and dilatation of capillaries

Cell overgrowth, which forms so conspicuous a feature of the destruction of the anterior part, is posteriorly absent in the beginning Only later, in advanced degeneration, foci of spindle-cells are seen between the tubules, around capillaries, and somewhat more abundantly at the periphery under the capsule It is not possible to state how far they may enter into the very last phases of destruction These are very difficult to determine, as they proceed with extraordinary rapidity In rabbits the whole disappearance is carried on from the nineteenth to the twenty-sixth day and the final solution of the posterior part between the twenty-first to the twenty-fifth day On the twenty-fifth day very little remains

During the early stages of the lesion, new tubules are said to be formed Felix attributes these either to scattered cells in the surrounding mesoderm or to budding of tubules and malpighian corpuscles With regard to this it may be stated that no evidences of budding of either tubules or malpighian corpuscles were seen in this series, although cell proliferation within tubules and glomeruli occurs

The lesions briefly described above attract attention on account of their similarity in character, genesis and distribution to certain types of degenerative and productive nephritis Indeed, so close is this relation that one may speak of an acute destructive mesonephritis

An investigation into its cause and significance seems, therefore, worth while

2 Three possibilities may be entertained in regard to the cause of mesonephric retrogression

A It may be due to crowding of the tubules within a small space To this has been attributed the early involvement of the most cranially situated tubules Felix, however, regards this as a very subordinate, if any, cause for the reason that the mesonephric arteries show at no time any indication of crowding by being bent in their course, but, on the contrary, remain perfectly horizontal There is, moreover, as seems to me, no histological evidence of crowding at any time, nor is the histogenetic picture of the retrogression itself in harmony with this view

B Mesonephric loss may be essentially due to interference and obliteration of its blood-supply This is also not borne out by the facts.

In the first place, the degeneration commences at a time when the mesonephric blood-supply is still quite adequate and sufficiently intact to allow the formation of new tubules In the second and most important place, the histological picture corresponds in no way to what is to be expected after quantitative interference with the blood-supply of an organ Two things may occur as the result of this, atrophy or infarction Here we have neither, but an extensive active cellular proliferation and invasion associated with a characteristically located rapid parenchymatous degeneration Both go far beyond the limits of the results of simple quantitative interference with the vascular supply of an organ Furthermore, the irregular streaky advance of the lesion, attacking certain parts while others are still intact, the capillary engorgement, degenerative cell waste and parenchymatous and interstitial cell proliferation leading to extensive cicatrization relate it to the inflammatory conditions

C There remains then the possibility of an influence which is conveyed to the mesonephros from other parts In this connection attention is immediately arrested by the peculiar relation in which growth and development of the medially attached sex gland stand to mesonephric destruction

They commence in closest association with each other and progress in inverse ratio Indeed, rapidity of growth and differentiation in one, and corresponding destruction and loss of the other are impressively associated In particular, the earliest and most pronounced changes leading to loss of malphigian corpuscles in the anterior part take place at the point of most intimate connection, and the degeneration appears to acquire momentum with progressing maturity of the genital gland We are confronted, therefore, by the question of a possible antagonistic influence of the latter on the mesonephros, and it is necessary to inquire how well such an idea is founded

3 The idea of antagonism between cells, tissues and organs is by no means new It has been emphasized by several biologists that among the forces which shape phylogenetic and ontogenetic evolution, antagonism between organs may be of as great an importance as correlation or altruism As early as 1876 Boll, in "Princip des Wachsthums," said

> Microscopic investigation has demonstrated in the peaceful egg the existence of highly dramatic processes which, out of compliment to a much-beloved terminology of doubtful value, I shall link together under the title of "the struggle of the tissues"[9]

9 Die mikroskopische Untersuchung hat im Frieden des Eies die Existenz hochst dramatischer Vorgänge nachgewiesen, welche ich aus Konzession an eine sehr beliebte Terminologie von zweifelhaften Werthe unter der Bezeichnung des *Kampfes der Gewebe* züsammenfassen will

It is well known that Roux has elaborated these ideas in his well-known monograph, "Der Kampf der Theile im Organismus," and recently von Hansemann, in "Deszendenz und Pathologie," has added another illuminating discussion of the subject The individual organism reflects to a great extent the forces which move a community of individuals In phylogeny, correlation and altruism of individuals are supplemented by a necessary antagonism to insure preservation and progress of the individual within the community, in ontogeny similar forces are repeated in the relation of cell to cell, tissue to tissue, organ to organ Thus weak and unnecessary organs which can no longer hold their own in the community of organs are removed, and the organism is freed from what would otherwise be a disadvantageous burden in its evolution We have certain reasons to suspect that on such antagonistic actions rest the disappearance of various embryonic structures, the thymus, for example, but in most instances this suspicion can only be vague The case under discussion, however, presents apparently a definite instance of growth and development of one organ at the expense of another How is this accomplished?

Goethe, St Hilaire and Roux have drawn attention to the fact that nutritive struggle plays an important rôle in mutual antagonism, for if one part receives for its growth a large supply of the available nutriment, others must suffer They termed this the law of compensatory growth Darwin, however, while admitting its importance, rather regarded it as a subordinate influence Roux believes, nevertheless, that such nutritional influences are by no means always of the ordinary direct mechanical circulatory nature, but of a much more complicated character He draws attention to the fact that women who nurse for years are very susceptible to osteomalacia because, he believes, of constant withdrawal of calcium salts in the milk, which is, therefore, unavailable for the bone The antagonism or, as he calls it, "struggle of the mammary glands with the bones," is characterized by greater strength of the former to attract and hold calcium salts Later investigators have endeavored to trace this to a direct influence of the sex gland It is possible that a nutritive struggle may enter into the antagonistic relations of mesonephros and sex gland But it must be considered doubtful whether it can be regarded as the sole cause of mesonephric loss, for certain features distinguish this from nutritive disturbances The mesonephros is apparently attacked by a distinctly irritative, destructive and, if the term is permissible, formative stimulus which leads to its speedy exitus

Again, in modern investigations many phenomena of organic interrelation are attributed to hormones Hormones may be defined as messengers from one organ to another which either excite or inhibit

functional activities The process under consideration can, therefore, hardly be grouped within the ordinary understanding of hormone action

Its exact nature remains unknown

Even if we were obliged to leave this point in abeyance, however, we may go further and ask whether the possibility of similar antagonism between organs may not outlast embryonic life and continue throughout postnatal existence Here we touch on points of great importance to the pathologist We are dealing in the embryo, as has been shown, with a mesonephritis which cannot be attributed to bacterial or an outside toxic influence It is the result of a true auto-intoxication We may, therefore, ask more specifically, whether similar phenomena continue after birth In other words, if, as seems highly probable, antagonism of organic units is one of the moving forces in the life of all individuals from practically the first division of the ovum to death, may it not enter more than hitherto supposed into production, manifestation and outcome of diseases? Perhaps etiology has for practical reasons been too anxious to attach an almost exclusive importance to external causes of disease, and has neglected a study of those internal conditions which prepare and make possible the development and specificity of morbid processes

The consideration of these problems reaches at present the borderland of pure speculation, but future investigations may put them, like many other at first sight phantastic views, on a sound disputable basis

Finally, we must record what is known of any possible function of the mesonephros in animals in which the renal activity has been handed over to the metanephros

Has the persistence of the mesonephros through at least part of the evolution of the individual any significance? This problem is intimately connected with the previous one

Johannes Muller, who discovered its capillary tufts, believed that the wolffian tubules secreted urine which passed through the wolffian duct into the cloaca or allantois

Mihálkovics came to the conclusion that the amniotic metanephros does not, in all probability, assume the functions of urine secretion suddenly or abruptly, as a considerable part of the wolffian body still exists at a time of advanced development of the metanephros There exists, in his opinion, a period when both organs functionate jointly as excretory organs Mihálkovics sought support of this contention in the phylogenetic history of the mesonephros, particularly in its continuation through the early part of postnatal life in reptiles This, in Mihálkovics' opinion, would indicate that among the lowest forms of amniotes the metanephros is assisted in some way and at least for a time by the mesonephros He concludes that in the evolution of amniotes there

existed antecedents, now extinct, in which the mesonephros persisted throughout life, while the metanephros remained incomplete and small This first anlage of the metanephros gradually developed through increasing and complicated demands to the size and importance of the present organ

Bonnet[2] also states that he has found urine in the mesonephric tubules of pig embryos commencing with 1 cm length and in the metanephric tubules in embryos from 8 cm length

In man the mesonephros acquires, as already emphasized, a considerable high state of differentiation There are, nevertheless, certain points which speak against its excretory activity in man Most important is in this respect its early degeneration, which, according to Felix,[2] commences even before the organ has fully developed Again, man lacks a vesicular allantois and urinary bladder in early life, and the urogenital sinus does not open to the outside until the embryo has reached a length of 14 mm (Bonnet) Bonnet states that the excretion of nitrogenous waste-products and elimination of water probably take place in the region of the allantois or by the chorionic circulation, and later by the umbilical vessels in the placenta It is, therefore, generally held that the mesonephros in mammals and particularly man possesses no excretory renal function

Is then the mesonephros a purely rudimentary organ which enters in no way into the welfare of the organism? Its very existence and high grade of differentiation would argue against it, for complete loss of function leads usually to either entire loss or a very incomplete appearance of an organ in later stages of phylogenetic evolution This, for example, is the case with the pronephros, whose very incomplete appearance in higher amniotes excludes almost with certainty the possibility of any function whatever

Following and guided by the history of some other organs of internal secretion, an effort has lately been made to find similar evidences for the mesonephros, but practically very little has been accomplished in this regard

The recent observations of Kermauner[10] offer perhaps a clue in this direction According to him the absence of the wolffian bodies is incompatible with life and leads to early death of the fetus He, therefore, regards the mesonephros of higher animals no longer as an organ of excretion, but of internal secretion (hormones) and assimilation Kermauner points in support of this idea to the rich blood-supply of the

10 Kermauner Das Fehlen beider Keimdrusen, Beitr z path Anat, von Ziegler (now Marchand and Aschoff), 1912, liv, No 3, p 478

Wolff, B Ueber foetale Hormone, in Handbuch der Biochemie von Carl Oppenheim, Erganzungsband, 1913

mesonephros with the numerous segmental arteries and veins which go to form its characteristic portal circulation

He concludes that the wolffian body is essential for certain periods of life, first in the development of the placenta, and secondly in complementing assimilative functions of other fetal parts until the appearance of permanent organs, notably the liver, renders this unnecessary The specific functions of the wolffian body are then no longer required, the fetus passes to new phases of life, and the mesonephros makes its exit

It must, however, be mentioned in this connection that the anlage of the mesonephric portal circulation in man is, according to Felix, only very short-lived, since in his observations the subcardinal veins and the transverse mesonephric branches are in act of degeneration in an embryo of 12 5 mm greatest length Kermauner's views are in their attempt of direct definition of mesonephric functions at the best very hypothetical, and need additional facts for sound basis On the other hand, it is of course well known that certain organs of internal secretion or specific metabolic activity[11] functionated originally as organs of external secretion They present, therefore, the peculiar phenomenon of functional mutation during phylogenetic evolution with morphological persistence Perhaps the thyroid gland furnishes the best example of this type, for whether we agree with Gaskell's extreme view that these glands were originally uterine, or whether we regard them, more conservatively, as derivatives of mucous glands,[12] the fact remains that they have undergone a functional mutation but have retained the acinar tubular structure which is characteristic of glands with an external duct

Two possibilities may exist in this transformation of glands of external secretion to glands of internal secretion and specific metabolic functions First, the glands possessed originally only the function of external secretion, and the others have been acquired or developed entirely *de novo* after the usefulness of the gland as an organ of external secretion has been ended

This would seem an unsatisfactory and unintelligible explanation, for it accounts in no way for the manner in which the new functions originated while structure and arrangement of the parts continued On the contrary, one would expect here as in other tissues that loss of function would go hand in hand with loss of structure or alteration in

11 I use the two terms "internal secretion" and "specific metabolic activity" because the influence of these glands may be due to other factors besides internal secretion Certain observations by Milne and Peters (Observations of the Glycolytic Power of the Blood and Tissues in Normal and Diabetic Conditions, Jour Med Research, 1912, xxvi, 415) make this probable for the pancreas

12 Gaskell See Footnote 2 For the evolution of the ductless glands consult Hertwig's Handbuch der Entwicklungsgeschichte

function with alterations in structure and rearrangement of parts This is the rule under physiological and pathological conditions

A second possibility seems, therefore, much more plausible and stands on some facts glandular organs possess from their origin functions of external and internal secretion and specific metabolic activities The exercise of these is initiated, developed and modified by the environment If thus, through evolutionary agencies, the necessity or even possibility for external secretion no longer exists, it ceases, but the gland still retains its functions of internal secretion and specific metabolism If now, further, no soil for this internal secretion has been developed,[13] or if the activity as a special metabolizer is no longer required, the gland is lost from disuse It is very possible that under such circumstances its removal is expedited by the antagonistic action of other glands If, however, a receptive soil for its internal secretion has been created and is retained during consecutive periods, or if its specific metabolizing function is adapted for succeeding evolutionary stages, the gland is not only retained, but strongly specializes under these stimuli in either one or both of these directions It may thus acquire an even greater influence on the internal economy of the organism than it formerly possessed with divided functions and, in turn, may play an important part in future evolution

We know, for example, that man still possesses several glands with external secretion which in addition are capable of and do exert strong internal influences, notably the testicle and the pancreas The older idea that these organs contained specific structures for internal secretion or special metabolic purposes has been replaced by the probably more correct view that the whole of the gland's parenchyma is potent in these regards This is certainly true of the pancreas,[14] probably also of the testicle, for while an internal secretion has been attributed to its interstitial cells, it seems certain that the glandular secretion which, moreover, is discharged only at relatively long intervals, exerts a very decided influence particularly on muscles and brain

13 In discussions of the disorders of internal secretion, perhaps insufficient attention has been paid to the importance which the soil may exercise in this connection It is evident, however, that the effect of an internal secretion depends not only on the function of the gland, but also on the condition of the particular soil which has become receptive to the secretion It would seem, therefore, that disturbances may arise not only through glandular disorders, but also through developmental or acquired faults of the soil This mutual relation may play an even greater part when close anatomical and physiological connections existed during embryonic life Finally, it seems not improbable that internal secretions may be modified on their way by passage through other glands This opens another opportunity for variations

14 Milne and Peters See Footnote 10 Potter and Milne Bronzed Diabetes, Am Jour Med Sc, 1911, cxlii, 46

The interesting and peculiar mutation of function with morphological persistence in glands is then rendered intelligible when we regard it as a relative one, representing rather a specialization than a new acquisition The rôle which these organs are to play in life and the duration of their existence are determined by first, the period of evolution at which the mutation occurs, secondly, the nature of their internal secretion or metabolism, and thirdly, their relationship to a particular soil or phase of general metabolism Consequently these organs become arranged according to periods of evolution, and appear and disappear with them Here as elsewhere ontogeny appears as a recapitulation of phylogeny

The mesonephros, although short-lived, may, nevertheless, exercise an influence on human fetal life and development not unlike that of other fetal and infantile structures It cannot, in view of what has been presented above, be denied But this question is, like the others, still far from being solved, and we possess to-day only two definite observations in this regard its complete and high degree of development and differentiation, and the statement advanced by Kermauner that its absence is not compatible with life A third, but much more uncertain, point might be construed from its relation to the genital gland This appears to owe its inception, growth and differentiation to the mesonephros, which it ultimately consumes Finally, a fourth, but equally uncertain conclusion, might be drawn from the histological pictures observed by Mihálkovics and Winiwarter and Sainmont These may indicate a secretory activity But we know from observations of the adult metanephros that such pictures are misleading[15] and not conclusive

These, then, are some of the problems which confront us in the study of renal evolution[16] That their bearing is not only of special, but also of very general pathological interest, seems not doubtful I enter these pages as a plea for their investigation and for a more careful consideration of ontogeny and phylogeny by pathologists

I have in conclusion many grateful acknowledgements to make Prof Arthur Keith, F R S, director of the Hunterian Museum at the Royal College of Surgeons, London, allowed me study of his specimens and put his technical assistant at my disposal Prof James P Hill, F R S, of University College, London, gave me free use of his collection of *Dasyurus viverrenus* and much advice To Prof Richard Assheton, F R S, of the University of Cambridge I am particularly indebted, not only for many carefully selected sections of rabbit embryos, but also for a great deal of active help and counsel Last, not least, to my colleagues in the Gordon laboratory, Guy's Hospital, Drs Laidlaw, Goodhart and Chisholm, I owe material and constant kind and helpful interest

29 St James' Street, S W

15 Aschoff, L Zur Morphologie der Nierensekretion, Verhandl d deutsch path Gesellsch, Jena, G Fischer, 1912

16 I have not touched at all in this communication on any possible differences in this process between male and female and, further, what determines the preservation and subsequent incorporation into the sexual system of certain mesonephric tubules These problems need special investigation

A METHOD DEVELOPED FOR OBTAINING A STANDARD WASSERMAN ANTIGEN *

CYRUS W FIELD, M D
NEW YORK

This method has been developed in the Pathological Department of Bellevue Hospital during the past six months as a result of a study on the routine Wasserman reactions of five thousand patients These patients have stayed in the hospital three days or longer, otherwise there has been no choice of subjects

For a long time it has been felt that the only variable reagent in the Wassermann reaction has been the antigen Antigens have been made up from the alcoholic extracts of human syphilitic livers, human hearts and ox-hearts The values of these various antigens have differed

TABLE 1—READINGS WITH THE SAME

Antigen	Serum A Antigen, Dil, c c							Serum B Antigens, c c							Serum C Antigens, c c						
	Control	1	0 8	0 6	0 4	0 2	0 09	Control	1	0 8	0 6	0 4	0 2	0 09	Control	1	0 8	0 6	0 4	0 2	0 09
Antigen 1	0	3	3	3	3	2	0	0	3	3	3	3	3	2	0	3	3	3	3	0	0
Antigen 2	0	3	3	3	3	3	0	0	3	3	3	3	3	1	0	3	3	3	3	1	0
Antigen 3	0	3	3	3	3	1	0	0	3	3	3	3	3	3	0	3	3	3	3	0	0
Antigen 4	0	3	3	3	3	2	0	0	3	3	3	3	3	2	0	3	3	3	3	3	0
Antigen 5	0	3	3	3	3	3	0	0	3	3	3	3	3	2	0	3	3	3	3	1	0
		High, 15, low, 13							High, 18, low, 16							High, 15, low, 12					

* In Tables 1 and 2, 0 = complete hemolysis, 1 = marked hemolysis, 2 = slight hemolysis Twenty-three other cases were 0 All these cases were specific as to history or lesion Forty other serums were negative

considerably in each batch made up This is apparently due to variations in the organs from single individuals For this reason the extract from guinea-pig hearts has been preferred because a larger number can be used for each batch of antigen, so that the individual factor is reduced to the vanishing-point

THE METHOD IN DETAIL

The method of conducting the Wassermann reaction in this laboratory is as follows

Patient's Serum —The patient's serum is poured off from the clotted blood and centrifuged The clear supernatant serum is removed and

* Submitted for publication March 3, 1914

inactivated at 56 C for half an hour A test-tube rack containing seven test-tubes is set up, and for the half quantity of the original Wassermann reaction 0 1 c c of the patient's serum is pipetted into each tube

Complement—The complement is obtained by bleeding at least three guinea-pigs and removing the serum after from twelve to eighteen hours This serum is diluted 1 part of serum to 8 of physiological salt solution, 0 5 c c of this diluted complement is added to each one of the seven tubes

Indicator—The indicator used to detect the presence or absence of the complement is 0 5 c c of a 5 per cent suspension of sheep-cells, to which has been added 0 5 c c of a dilution of amboceptor of such strength that each cubic centimeter contains four hemolytic units This mixture is allowed to incubate at 37 C for half an hour before being added to the Wassermann system It has been found from a study of over ten thousand Wassermann reactions that the native amboceptor occurring in human blood may be disregarded if this be done

SERUMS WITH FIVE DIFFERENT ANTIGENS*

Control	Serum D Antigens, c c 1	0 8	0 6	0 4	0 2	0 09	Control	Serum E Antigens, c c 1	0 8	0 6	0 4	0 2	0 09	Control	Serum F Antigens, c c 1	0 8	0 6	0 4	0 2	0 09	Control	Serum G Antigens, c c 1	0 8	0 6	0 4	0 2	0 09
0	3	3	3	3	0	0	0	3	2	1	0	0	0	0	3	3	3	3	3	0	0	3	0	0	0	0	0
0	3	3	3	2	0	0	0	3	3	3	0	0	0	0	3	3	3	3	3	0	0	2	0	0	0	0	0
0	3	3	3	3	0	0	0	3	3	2	0	0	0	0	3	3	3	3	2	0	0	3	1	0	0	0	0
0	3	3	3	2	0	0	0	3	3	1	0	0	0	0	3	3	3	3	3	0	0	3	0	0	0	0	0
0	3	3	3	1	0	0	0	3	3	3	0	0	0	0	3	3	3	3	1	0	0	2	1	0	0	0	0
High, 12, low, 11							High, 9, low, 7							High, 15, low, 13							High, 4, low, 2						

and 3 = no hemolysis

Antigen—The antigen that is now used is an alcoholic extract of guinea-pig hearts to which has been added enough cholesterin to bring it to the half-saturation point When the pigs are killed the heart is removed, and all blood present in the cavities is removed by massaging with a clean towel The heart is weighed, this weight being recorded on the jar in which the heart is preserved until a sufficient amount has accumulated In preparing this antigen at least 300 gm of guinea-pig heart, after having been finely ground, is extracted for fourteen days at 37 C with 3,000 c c of absolute alcohol, chemically pure It is then filtered, and half the filtrate is placed in a flask and an excess amount of chemically pure cholesterin is added It is allowed to stand in the incubator over night The next morning the flask is removed from the

incubator and placed in a constant temperature bath at 16 C for three hours This is to precipitate the excessive amount of cholesterin It is now filtered and this saturated cholesterin solution is added to an equal quantity of the filtered alcoholic extract Twenty cubic centimeters are removed with a pipet and placed in a weighed dish and evaporated at 56 C. to dryness It is then placed in a desiccator for twenty-four hours over sulphuric acid and then weighed It is thus determined what the total lipoid cholesterin concentration of this alcoholic solution is After having determined this, it is tested for its anticomplementary effect

In all, five different lots have been made up by this method, and they have been remarkably constant in their total lipoid concentration and their anticomplementary value, and, as will be seen from Table 1, in their antigenic value

Dilution for Use.—One cubic centimeter of the alcoholic extract is added to as many cubic centimeters of salt solution as is necessary to make the lipoids and cholesterin weight equal to 0 1 mg per cubic centimeter That is, if 100 c c of the alcoholic extract contain 0 780 gm, we add 1 c c of this to 77 c c of physiological salt solution This is the first antigen dilution, and 1 c c is placed in the second tube in the rack (the first tube containing only salt solution and no antigen, this being the control) In the third tube is placed 0 8 c c of this antigen dilution, in the next 0 6, in the next 0 4, in the next 0 2, and in the next 0 09 This gives us a series of antigen tubes with decreasing amounts of antigen and a constant amount of serum Enough 0 8 per cent sodium chlorid solution is added to each tube to make the total volume 1 6 c c The antigen can be diluted with the sodium chlorid solution before it is added to the tube, this is perhaps easier to do The reading is made as soon as a control set of tubes containing complement and the various strengths of antigen, and the control tube in the individual set, show complete hemolysis

Only four degrees of hemolysis are observed in the tubes zero, equaling complete hemolysis, 1, marked hemolysis, 2, slight hemolysis, and 3, no hemolysis In this way, having three degrees of inhibition for each tube and six antigen tubes, we have a total possibility of eighteen units, provided complete inhibition occurs in all these tubes Less than 0 5 per cent of all positive cases give eighteen or more units

Duplicate tests of patient's serum, unknown as duplicates to the person who made the readings, came out within one or two units of each other constantly The tests on the five different batches of antigen made up with this procedure have shown no greater differences with the same serum, that is, one or two units This is a much more constant reading than we have been able to obtain with any antigens heretofore With

this method the antigen is practically as constant in quality as is the amboceptor or complement

Table 1 shows a series of readings with the same serums with the five different antigens Table 2 shows duplicate serum readings with one of the antigens

TABLE 2.—DUPLICATE SERUM READINGS WITH ONE OF THE ANTIGENS

Serum	Control	1	0 8	0 6	0 4	0 2	0 09	Units of Serum	Units in Sp Fl
J K	0	3	3	3	3	3	0	15	15
	0	3	3	3	3	3	0	15	
T R	0	3	3	3	3	2	0	14	
	0	3	3	3	3	3	0	15	
P C	0	3	3	3	3	3	2	17	15
	0	3	3	3	3	3	2	17	
J L	0	3	3	3	3	3	2	17	
	0	3	3	3	3	3	1	16	
M	0	3	3	3	0	0	0	9	
	0	3	3	2	0	0	0	8	

CONCLUSIONS

1 The cholesterin, guinea-pig heart antigen is readily obtained and constant in its antigenic value (This has already been suggested by Swift and Ellis and others)

2 A series of dilutions of the antigens is obtained by weight, which has been shown to give constant results

3 A Wassermann reaction may now be standardized so that the readings will agree very closely in far distant laboratories

4 In the use of this antigen the patient's serum must be inactivated

5 There must be more than two units of complement

6 The red cells must be sensitized by the amboceptor before being added to the system

This antigen has been criticized as being too delicate and as giving positive results in non-specific cases

More than five thousand cases were run in duplicate, one set with this antigen and the other with an acetone-insoluble antigen from ox-heart There were 8 per cent positive with the cholesterin guinea-pig heart which were negative with the ox-heart, but in none of them was it possible to exclude syphilis, in fact, the vast majority of them showed luetic infection or the results of such an infection, or had a positive history

A more detailed paper will be published later, giving the results of this method as applied to treatment and in the individual variations of the Wassermann reaction in the same individual over various periods

Pathological Laboratory, Bellevue Hospital, Twenty-Ninth Street and First Avenue

A STUDY OF THE ACTION OF ATROPIN ON THE EOSINOPHIL CELLS OF THE BLOOD *

W W HERRICK, M D
NEW YORK

Our knowledge of the functions of the eosinophil cells is slight The most important and perhaps the only well-demonstrated function of these cells is their association with the reaction of the body to the invasion of alien protein The notable rise in number of eosinophils in the circulating blood following the parenteral introduction of various proteins is now well known By the work of Schlecht,[1] Schlecht and Schwenker,[2] Schittenhelm, Weichardt and Grisshammer,[3] Staubli,[4] Herrick,[5] and Ahl and Schittenhelm[6] it has been thoroughly established that the subcutaneous, intraperitoneal or intravenous introduction of alien proteins at longer or shorter intervals is followed by a definite eosinophilia of the blood Particularly is this a feature of the post-anaphylactic stage following the injection of protein at intervals of five days or more The eosinophilia following administration of diphtheria antitoxin and of tuberculin has been recognized for a still longer time Possibly the eosinophilia observed during convalescence from various infections is a manifestation of the same response on the part of the living organism to the invasion of foreign protein

Since the problem of immunity is bound up with that of the adaptation of the body to alien protein material, and since the eosinophils have such close relation to the process, any study throwing light on the

* Submitted for publication March 3, 1914

* From the Department of Practice of Medicine, College of Physicians and Surgeons, Columbia University, New York

1 Schlecht Ueber die Einwirkung von Seruminjectionen auf die Eosinophilen und Mastzellen des menschlichen und tierischen Blut, Deutsch Arch f klin Med, 1910, xcviii, 308

2 Schlecht and Schwenker Ueber die Beziehungen der Eosinophilie zur Anaphylaxie, Deutsch Arch f klin Med, 1912, cviii, 405

3 Schittenhelm, Weichardt and Grisshammer Eiweissumsatz und Ueberempfindlichkeit, Ztschr f exper Path u Therap, 1912, x, 3, 412, Ueber den Einfluss parenteral verabreichter Proteinsubstanzen verschiedener Herkunft auf das Blutbild, ibid

4 Staubli Die klinische Bedeutung der Eosinophilie, Ergebn d inn Med u Kinder, 1910, vi, 12, Ueber Eosinophilie, Samml klin Vortr, 1909, clxvii, 43

5 Herrick, W W Experimental Eosinophilia with an Extract of an Animal Parasite Its Relation to Anaphylaxis and Certain Clinical Problems, THE ARCHIVES INT MED, 1913, xi, 165

6 Ahl and Schittenhelm Ueber experimentelle Eosinophilie nach parenteraler Zufer verschiedener Eiweissstoffe, Ztschr f d ges exper Med, 1913, i, 111

functions of these important cells offers at least the chance of illuminating the larger problem. It has been with this possibility in mind that the study of the effect of atropin on the eosinophils was undertaken.

Ehrlich, cited by Mayer,[7] has remarked that too little experimentation has been done on the relation of the eosinophils to chemical substances, and that the results thus far obtained have been inconstant because insufficient material has been used in such experimentation. In the present study of the influence of a chemical substance on the eosinophils it is thought that sufficient material has been used to meet this very appropriate objection.

Atropin was not selected at random, neither is the problem the narrow one that would at first thought appear. Auer[8] has recorded the observation that anaphylactic shock in guinea-pigs may be prevented by the injection of from 0.5 to 1 mg. of atropin. Since eosinophilia so constantly follows recovery from anaphylaxis, it is of interest to learn whether or not atropin influences the cellular reaction. Again, a clinical problem is concerned in the question of the control of the eosinophils by atropin. The term "eosinophil diathesis" has found its way into medical terminology. Under this heading are classified those persons prone to bronchial asthma, mucous colitis, urticarias and allied conditions that have as a frequent accompaniment an eosinophilia of the blood. Eppinger,[9] in a description of the somewhat vague clinical concept called "vagotonie," states that cases characterized by excessive functioning of the autonomic nervous system, "vagotonikers," show an excess of eosinophils in the circulating blood, which excess can be reduced by atropin. No experiments are cited in support of this statement. It has been suggested elsewhere that there is a relation between the problems of anaphylaxis, eosinophilia and that of bronchial asthma, that substances capable of influencing the eosinophil cell might be of clinical use in dealing with that baffling symptom asthma. With these questions in their somewhat complex relations in view, the study of the influence of atropin on the eosinophils was undertaken.

METHODS OF STUDY

Guinea-pigs were chosen for experiment for reasons sufficiently explained in numerous other communications by various writers.[10] The

7 Meyer: Die klinische Bedeutung der Eosinophilie, Berlin, S. Karger, 1905.

8 Auer: The Effect of Vagus Section on Anaphylaxis in Guinea-Pigs, Jour. Exper. Med., 1910, xii, 638.

9 Eppinger and Hess: Die Vagotonie, Samml. klin. Abhandl., 1910, Nos. 9 and 10, p. 41. Hopkins: A Clinical Study of Vagotonia, THE ARCHIVES INT. MED., 1913, xii, 556.

10 For discussion of the blood-picture and blood reactions of the guinea-pig the following may be consulted:

Opie: The Occurrence of Cells with Eosinophil Granulations, and Their Relation to Nutrition, Am. Jour. Med. Sc., 1904, cxxvii, 217. An Experi-

blood-counts were made by the method most commonly in use in clinical work Blood was taken from the ear The usual pipet giving a dilution of 1 10 was used to determine the total number of leukocytes, while for the differential count smears were made, stained by Wright's stain, and 200 cells counted with the oil-immersion lens Several control counts were made before beginning each experiment In all ten guinea-pigs were used Atropin was given in a 10 per cent solution by hypodermic injection The horse-serum was given intraperitoneally The dates of the blood-counts, and the amounts and times of administration of atropin and of serum are detailed in the accompanying charts, together with the curve illustrating the variations in total numbers of the eosinophil cells in a cubic millimeter of blood The dosage of atropin was large, allowing for the tolerance to this drug shown by the graminivora

PROTOCOLS OF EXPERIMENTS

EXPERIMENT 1.—Guinea-Pig 1 weighed 640 gm Control counts showed constant eosinophilia, from 817 to 4,867 in a cubic millimeter From June 27 to July 23 eleven doses of atropin sulphate, 0 002 gm, were given From July 29 to August 13 ascending doses of from 0 04 to 0 15 gm were given Exact amounts

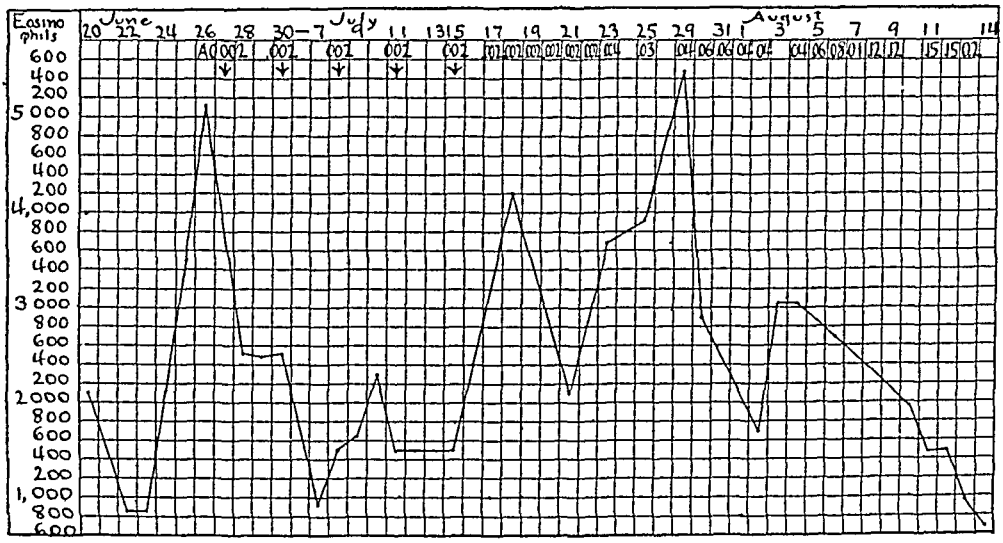


Chart 1.—Experiments on Guinea-Pig 1 In the charts the atropin dosage is given in grams, the figures preceded by the letter "A," and the serum is given in cubic centimeters, the figures preceded by the letter "S"

mental Study of the Relation of Cells with Eosinophil Granulation to Infection with an Animal Parasite, ibid, 1904, cxxvii, 477

Staubli Trichinosis, 1909, p 107 et seq

Gulland and Goodall The Blood, 1912, p 76

Kanthack and Hardy Wandering Cells of Mammalia, Jour, Physiol, London, 1894, xvii, 81

Herrick, W W Footnote 5

Klineberger and Karl Die Blutmorphologie der Laboratoriumstiere, Leipsic, 1912

and times of administration may be learned from Chart 1. There was definite fall in the eosinophil curve during the last ten days of the experiment, the period of administration of huge doses of atropin. At the same time there were toxic symptoms: loss of weight, roughening of the coat, weakness, loss of appetite, lessened activity, etc. Weight August 14, 592 gm.

EXPERIMENT 2.—Guinea-Pig 2 weighed 670 gm. Control counts showed eosinophils varying in numbers from 1,950 to 5,340. From June 30 to July 22, ten doses of atropin sulphate 0.002 gm., were given on alternate days without influence on the numbers of these cells. From July 23 to August 12, atropin sulphate in amounts from 0.04 to 0.15 gm. were given daily or every second day. No notable fall in the eosinophil curve was apparent. Toxic symptoms were

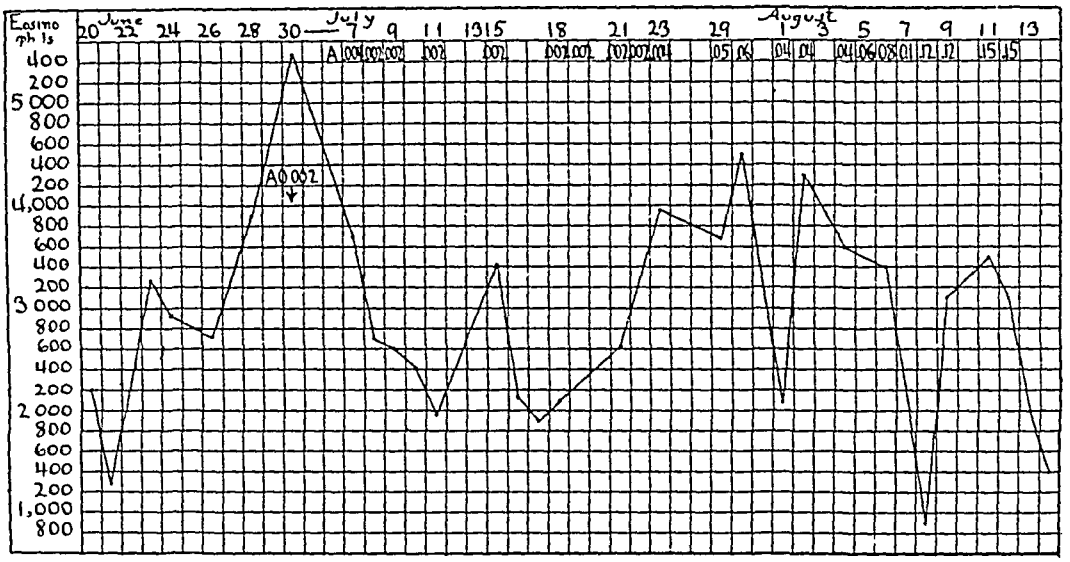


Chart 2.—Experiments on Guinea-Pig 2.

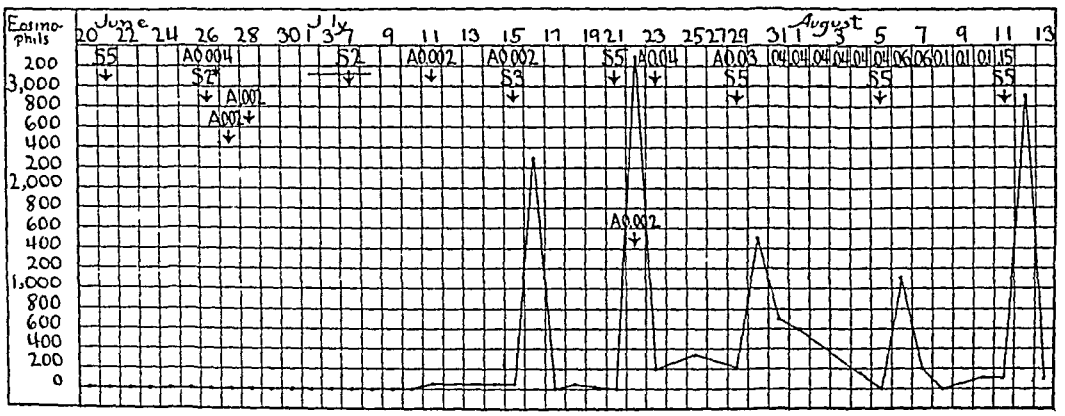


Chart 3.—Experiments on Guinea-Pig 3. * June 26, the serum was given one hour after the atropin.

produced of a character similar to those described in the case of Guinea-Pig 1. Weight August 13, 618 gm.

EXPERIMENT 3.—Guinea-Pig 3 weighed 500 gm. During the control period no eosinophils were found in the circulating blood. Interval injections of serum after the third were followed by the usual eosinophilia. Atropin in large amounts, from 0.03 to 0.15 gm. on the day before that of the injection of serum apparently inhibited the rise in number of eosinophils in two instances, but in one instance

did not prevent the development of a marked eosinophilia, which occurred despite daily injections of large amounts of atropin for a period of twelve days The results in this instance are therefore contradictory

EXPERIMENT 4.—Guinea-Pig 4 weighed 480 gm The eosinophil response on the part of this animal to repeated injections of serum was slight though definitely present This may have been due to the apparent general feebleness of the animal, which succumbed to a dose of atropin sulphate, 0 1 gm Atropin given in doses of 0 002 or of 02 gm at the time of administration of serum had no apparent effect on the subsequent rise in number of eosinophils

EXPERIMENT 5.—Guinea-Pig 5 weighed 500 gm This animal was used as a control to show the effect of interval injections of serum on the eosinophils Atropin sulphate, 0 04 gm., given at the time of the sixth inoculation of serum, apparently exerted an inhibitory influence on the subsequent eosinophilia This eosinophilia, though present, was much less than in the two preceding instances following inoculation of serum alone, and may possibly have been a result of the action of atropin

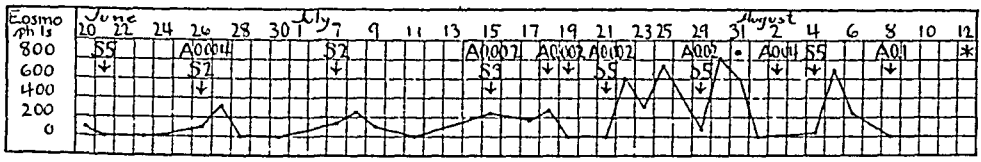


Chart 4.—Experiments on Guinea Pig 4 * Ill, weak, died

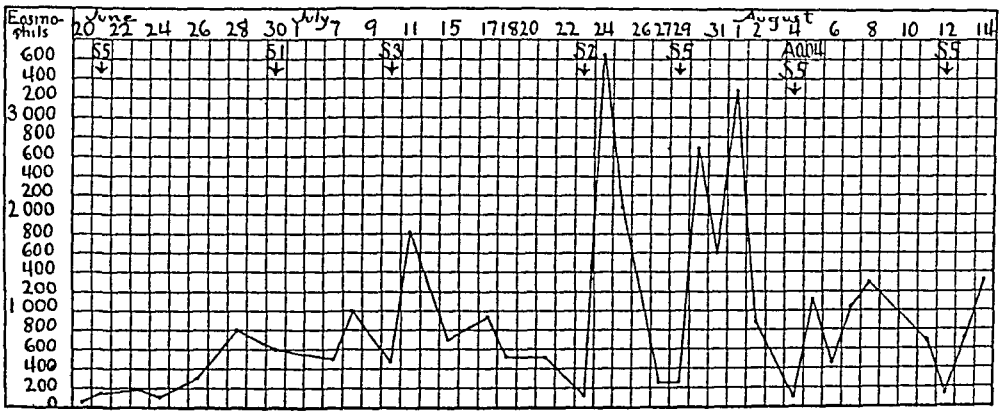


Chart 5.—Experiments on Guinea-Pig 5

EXPERIMENT 6.—Guinea-Pig 6 weighed 480 gm Control counts of eosinophils showed from 270 to 648 Three injections of serum alone at intervals of from seven to twelve days were followed in case of the last two by increase of eosinophils, the maximum number being 4,638 July 29, atropin sulphate, 0 02 gm., with serum, 5 c c, was injected There was response on the part of the eosinophils, their numbers rising from 836 to 2,362 forty-eight hours later August 4, atropin, 0 04 gm., was injected August 5, serum, 5 c c, was injected Eosinophils, absent on the 5th, rose to 1,120 the 6th, a much lower figure than that seen after other injections of serum alone in this animal August 11, eosinophils were 576 Atropin, 0 1 gm was injected August 12, eosinophils were 1,581 Serum, 5 c c was injected August 14, eosinophils were 864 In this instance the usual rise in the number of eosinophils was lacking

EXPERIMENT 7.—Guinea-Pig 7 weighed 500 gm In this animal enormous and finally lethal doses of atropin did not prevent the development of the eosinophilia

consequent on the interval injections of serum, such an eosinophilia being present on the day of death

EXPERIMENT 8.—Guinea-Pig 8 weighed 560 gm. Control counts showed a moderately high eosinophilia, averaging 1 300 in a cubic millimeter. Atropin sulphate, 0 05 gm., was given twice daily from December 1 to 9. On the latter date a single dose of 0 1 gm. was given. From November 15 to December 9 four interval injections of serum, 5 c c., were given, the last injection being followed within two minutes by convulsions and death. It is of interest that

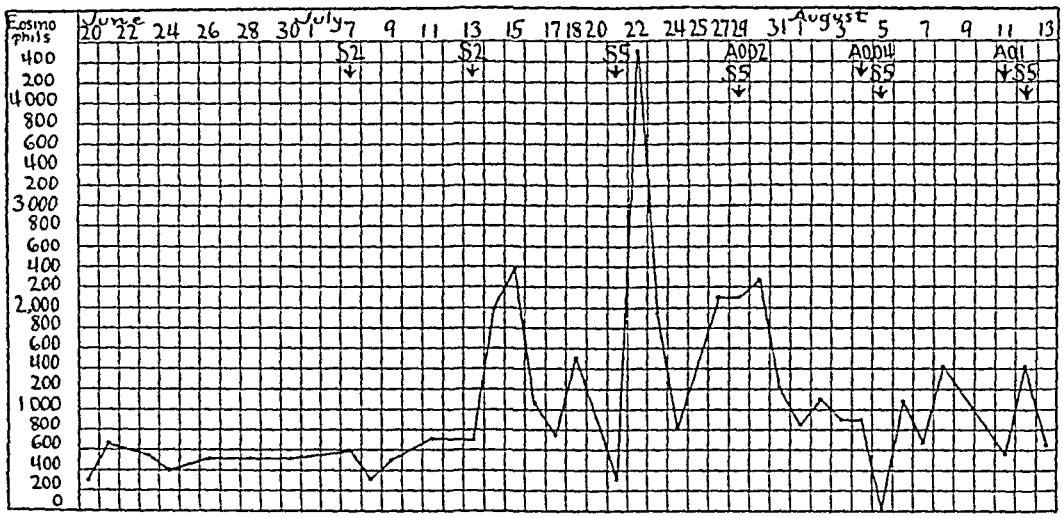


Chart 6.—Experiments on Guinea-Pig 6

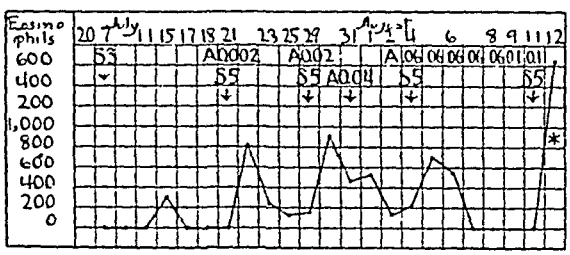


Chart 7.—Experiments on Guinea-Pig 7. * Died.

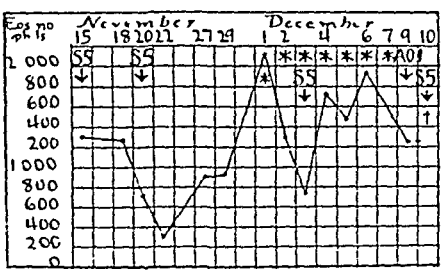


Chart 8.—Experiments on Guinea-Pig 8. * Atropin sulphate, 0 05 gm. twice daily. † Death in two minutes from convulsions.

the large amounts of atropin administered before the fatal injection of serum did not prevent the development of an apparent acute anaphylaxis. The injections of serum were not followed by any notable rise in the number of eosinophils. Throughout the period of administration of atropin and serum the average level of the eosinophil curve remained about as during the control period. December 8 the weight was 543 gm.

EXPERIMENT 9.—Guinea-Pig 9 weighed 521 gm. Control counts showed constant eosinophilia, these cells numbering from 1,000 to 2,000 in a cubic millimeter. Serum, 5 c.c., was given November 15. This was repeated November 20 and was followed by a pronounced rise in eosinophils to 1,825. Atropin, 0.05 gm., was given twice daily from December 1. Serum, 3 c.c., was given December 3 without subsequent eosinophilia. December 10, serum, 5 c.c., was injected. No eosinophilia followed, and on December 11 and the following days these cells diminished in number. A fifth injection of serum, December 16, was followed by a very slight rise in the eosinophils, from zero to 500. In this instance there was definite effect on the eosinophils, the diminution in their numbers after the administration of atropin being constant and striking. The animal showed toxic symptoms and lost weight to 493 gm.

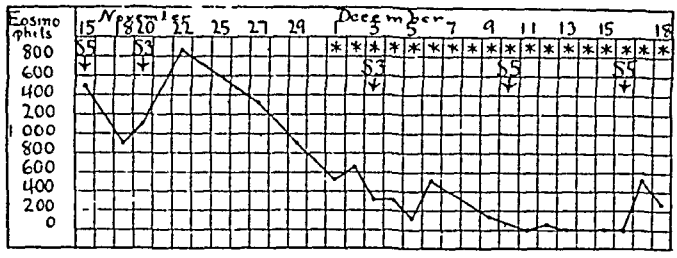


Chart 9.—Experiments on Guinea-Pig 9. * Atropin sulphate, 0.05 gm. twice daily.

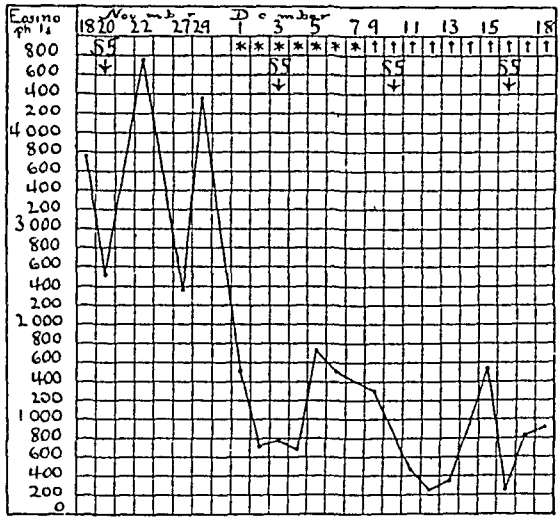


Chart 10.—Experiments on Guinea-Pig 10. * Atropin sulphate, 0.05 gm. twice daily. † Atropin sulphate 0.1 gm. twice daily.

EXPERIMENT 10.—Guinea-Pig 10 weighed 522 gm. Control counts of eosinophils were high—from 2,400 to 4,800. From December 1, atropin, 0.5 gm., was given twice daily. After this date the eosinophils at no time numbered more than 1,700. From December 9 the amount of atropin given was doubled, 0.1 gm. being the dose administered. Following this the eosinophil count fell to 200, and remained constantly low. Interval injections of serum resulted in no significant eosinophilic increase, in fact, in one instance a fall followed such an injection. The effect of atropin seems definite in the case of this animal. There were toxic symptoms and loss of weight to 490 gm.

REVIEW OF EXPERIMENTS

Review of the experiments detailed above brings out the fact that definite fall in the number of eosinophils followed administration of atropin in but two of ten animals The animals in which such positive result was obtained were two of three receiving large doses of atropin, from 0 05 to 0 1 gm , every twelve hours (Guinea-Pigs 9 and 10) The third animal receiving such doses showed no effect on the eosinophils (Guinea-Pig 8) This guinea-pig, however, died from anaphylaxis before a sufficient period of observation had elapsed to make conclusions of value A less definite result was seen in Guinea-Pig 1 This animal received daily doses of atropin from 0 03 to 0 2 gm , and showed considerable reduction in the number of eosinophil cells from the high average found throughout the earlier part of the period of observation Guinea-Pig 2, on the same dosage of atropin, showed little change in number of eosinophils The smaller doses of atropin, from 0 002 to 0 004 gm , were without influence on the eosinophil content of the blood In order, therefore, to reduce a high eosinophilia with atropin, very large doses sufficient to cause toxic symptoms must be given at frequent intervals

INFLUENCE OF ATROPIN ON THE EOSINOPHILIA FOLLOWING INJECTION OF SERUM

Atropin given with, immediately before or twenty-four hours before the interval injection of serum, seemed to inhibit to some degree the subsequent rise in number of the eosinophils (Guinea-Pigs 3, 5, 6, 9 and 10) , such rise being absent or less than that occurring in the same animal after injections of serum without atropin This result is not constant For exceptions observe Charts 3, 4 and 7 In animals receiving large doses of atropin at intervals of twelve hours, eosinophilic response was slight or lacking (Guinea-Pigs 8, 9 and 10) Frequently there was a drop in the curve of these cells after such injections—a most exceptional occurrence under ordinary conditions

DISCUSSION OF RESULTS

It seems clear that the mechanism by which the number of eosinophils in the circulating blood of guinea-pigs is increased is not influenced by ordinary amounts of atropin This mechanism, however, may be depressed or paralyzed by excessive doses of the drug, when such doses are given as often as every twelve hours

The method of action of atropin in this connection is obscure and involves the question of the general control of leukocytes, which is far from solution One can only speculate as to the possible influence of atropin on chemotaxis, or as to an altogether hypothetical influence of

the autonomic nervous system on bone-marrow activity which is inhibited by the alkaloid, or one may, perhaps with more reason, consider that the results noted are due to a general interference with a highly specialized function by a strong poison and not to any more specific influence In connection with the latter conception, Opie's demonstration of the close relation of the number of eosinophils of guinea-pigs to their nutrition is important Opie[10] found that in guinea-pigs in which there was loss in weight from starvation or other cause the eosinophil cells were regularly much reduced in number In the present study the nutrition of the animals suffered from the large doses of atropin Loss of weight and appetite, roughening of the coat, lessened activity, etc, were noted in all animals showing reduction in numbers of eosinophils or lack of response on the part of these cells to serum injections It may therefore be reasonable to consider the effect of atropin on the eosinophils here observed an indirect one and a result of disturbed nutrition from large doses of a poisonous alkaloid

There is strong objection to applying by analogy to man conclusions gained by experiments on an animal like the guinea-pig, particularly with atropin, a poison to which the animal is, as compared to man, relatively tolerant The very large doses of atropin given, however, and the fact that the blood reactions of the guinea-pig are strikingly like those of man, must have weight Clinically it has never been possible in my experience notably to reduce a hypereosinophilia by atropin in ordinary doses Animal experimentation seems to lend some degree of corroboration to this clinical experience

CONCLUSIONS

Atropin in ordinary, non-toxic doses has no striking influence on the eosinophil cells of the guinea-pig's blood

Given in toxic doses resulting in loss in weight and other signs of disturbed nutrition, and particularly when such doses are repeated as often as every twelve hours, there is a diminution in the number of these cells, and the eosinophilia following interval injections of serum is diminished or absent

It is probable that this effect of atropin is not specific, but is the depression of a highly specialized function by the general effect on nutrition of large amounts of a strong poison

CLINICAL OBSERVATIONS ON DEFECTIVE CONDUCTION IN THE BRANCHES OF THE AURICULO-VENTRICULAR BUNDLE *

A REPORT OF TWENTY-TWO CASES, IN WHICH ABERRANT BEATS WERE OBTAINED

EDWARD PERKINS CARTER, M D
CLEVELAND, O

The present communication is based on material collected in the Cardiographic Department of University College Hospital Medical School, London It includes a description of twenty-two cases in which evidence of defective conduction in one or the other branch of the auriculoventricular bundle was observed electrocardiographically, a description of the characteristic features of aberrant curves and remarks on the clinical associations of the corresponding defects

As defective conductivity of the main stem of the auriculoventricular bundle gives rise to a characteristic electrocardiogram, so do lesions of its individual branches give rise to abnormal electrocardiographic curves, which, within certain limits, follow definite types It is with these definitely known forms of abnormal curves that we are concerned

As *aberrant*[1] are grouped those heart-beats which result as a direct sequence of impulses which arise in a supraventricular focus, but which reach the ventricle either through an unusual route or through normal but partially defective channels In either case the resulting electrocardiograms will show conspicuous differences from the normal, differences which depend either on a permanent organic lesion, or, less commonly, on a temporary defect in conductivity

By supraventricular focus is meant a focus in the auricle or junctional tissues above the division of the main stem of the bundle The impulses giving rise to the independent ventricular rhythm of complete heart-block are of supraventricular origin, for they arise in the main bundle, and so, as we shall see, aberrant types of ventricular contraction may be associated with complete dissociation

It has been shown by Eppinger and Rothberger[2] that experimental lesions of the branches of the auriculoventricular bundle are followed by characteristic electrocardiographic curves, that a lesion of the right

* Submitted for publication Feb 19, 1914

1 Lewis Heart, 1912, iii, 293

2 Eppinger and Rothberger Ztschr f klin Med, 1910, lxx, 1

branch of the auriculoventricular bundle gives rise, in Lead II (Rt A — Lt L), to an electrocardiogram which resembles that obtained on stimulation of the left and apical portion of the ventricle, the initial deflection of the ventricular complex being directed downward (apex negative), while a lesion of the left branch of the auriculoventricular bundle gives rise to the reverse picture, an electrocardiogram resembling that obtained on stimulation of the right and basal portion of the ventricle, the initial deflection of the ventricular complex being directed upward (base negative)

In either instance the resulting electrocardiogram is essentially diphasic, and, although always preceded by an auricular complex, the ventricular complexes resemble those known to occur as the result of intrinsic premature ventricular contractions, as shown by the prolongation of the period covering the opening phases, by the increase in amplitude and direction of the initial deflection in the several leads Soon this experimental evidence was further established by Eppinger and Stoerk,[3] who were able, in two clinical instances by subsequent post-mortem examination, to confirm the diagnosis made from electrocardiograms of a lesion of the right branch of the auriculoventricular bundle

More recent clinical and electrocardiographic evidence has confirmed these earlier observations, a number of workers having reported in detail cases associated with lesions of one or the other branch of the auriculoventricular bundle, in some of which the diagnosis has been confirmed by histological examination

Cohn and Lewis[4] report a most unusual case of auricular fibrillation and heart-block, in which it was shown by a histological examination that both branches of the auriculoventricular bundle had been destroyed The electrocardiograms obtained from this case were practically identical with the curves obtained from a similar experimental lesion by Eppinger and Rothberger

Lewis[5] has published a report of a unique case illustrating a temporary defect in conductivity of the right branch of the auriculoventricular bundle, the records of which are included in the present series (Case 12)

Mathewson[6] has recently reported four cases associated with lesions of one or the other branch of the auriculoventricular bundle

In the light of the accumulated observations it has seemed possible to attempt to classify in greater detail the aberrant curves resulting from lesions of the branches of the auriculoventricular bundle

3 Eppinger and Stoerk Ztschr f klin Med, 1910, lxx, 157
4 Cohn and Lewis Heart, 1912, iv, 15
5 Lewis Brit Med Jour, 1913, i, 484
6 Mathewson Heart, 1913, iv, 385

DISTINCTIONS BETWEEN PHYSIOLOGICAL AND ABERRANT TYPES OF CURVE

In order to bring out more clearly the essential distinctions between the normal and the aberrant electrocardiogram, I summarize in tabular form the chief points of difference between them and physiological electrocardiograms It must be borne in mind, however, that, though we are dealing with an aberrant type of electric curve, the ventricular contractions were of supraventricular origin, each ventricular beat being preceded by an auricular contraction—except in one case of complete heart-block—and that the resulting ventricular complexes alone are abnormal

In the accompanying figures all three leads have been employed, as it is possible to arrive at accurate conclusions only by an analysis of the curves obtained from the three leads In Lead II, in the presence of lesions of the branches of the auriculoventricular bundle, the initial deflection of the ventricular complex usually follows the direction of the corresponding deflection in Lead III, but there are exceptions to this rule

TABLE 1.—CONTRASTS BETWEEN NORMAL AND ABERRANT ELECTROCARDIOGRAMS

NORMAL	ABERRANT
1 Supraventricular complex Presence of auricular or *P* summits	1 Supraventricular complex Presence of auricular or *P* summits
2 *P-R* interval 13 to 18 second, never more than 0 2 second	2 *P-R* interval frequently prolonged beyond 0 2 second
3 *Q R-S* interval less than 0 1 second, and less than one third of entire complex	3 *Q-R-S* interval exceeds 0 1 second and as a rule constitutes more than one third of the entire complex
4 Relatively small amplitude of initial deflections	4 Relatively increased amplitude of initial deflections
5 Final deflection *T* upright and in the same direction as the most prominent deflection (*R*) in Leads I and II, and usually in Lead III	5 Final deflection *T′* usually in a direction opposite to that of the prominent initial deflection May be upright or inverted
6 Initial deflections as a rule unnotched	6 Initial deflections almost always show notching in one lead at least Many bizarre forms seen
7 Final deflection *T* as a rule plainly to be seen, but not exaggerated	7 Final deflection *T′* frequently much exaggerated

Thus it will be seen that the differences between the normal and the aberrant electrocardiogram consist essentially in the longer duration of the opening phases, in the greatly increased amplitude of the initial deflections, in the notching of *R′* and *S′* so often seen in the aberrant curves—the initial deflections are spoken of as *R′* and *S′*, to distinguish them from the deflections *R* and *S* of the normal curve—and in the generally diphasic character of the aberrant curve, especially in Leads I and III, while the normal electrocardiogram is triphasic or quadriphasic

Whatever the location of the lesion, whether involving the right or the left branch of the auriculoventricular bundle, the prominent initial deflections are always in *opposite* directions in Leads I and III This rule, while constant for R' and S', usually holds for the final deflection T', which, as has been stated, commonly lies in a direction opposite to the prominent initial deflections R' and S', and is usually conspicuous

Figure 8 illustrates in detail the distinguishing characteristics of the aberrant electrocardiogram, it is an instance of temporary defect in the conductivity of the right branch of the auriculoventricular bundle, associated with relative left ventricular hypertrophy The greatly increased duration and amplitude of the initial deflections is at once apparent The P-R interval measures 0 18 sec The Q-R-S interval measures more than 0 1 sec The conspicuous initial deflections R' and S' in Leads I and III are seen to be in opposite directions The initial deflection S' in Lead II follows the same direction as the corresponding deflection in Lead III The final deflection T' is in a direction opposite to the conspicuous initial deflection in all leads, being inverted in Lead I, and upright in Leads II and III The notching of R' in Lead I and of S' in Leads II and III is apparent The final deflection T' is much exaggerated

DISTINGUISHING POINTS BETWEEN THE ABERRANT ELECTROCARDIOGRAM AND THAT OBTAINED AS A RESULT OF CARDIAC HYPERTROPHY

While it is freely admitted that the attempt to correlate certain types of electrocardiogram with a definite form of cardiac hypertrophy may need subsequent revision, it has been shown that in the presence of specific valve-lesions, commonly associated with preponderating hypertrophy of the left or right ventricle, distinctive electrocardiograms have been obtained, controlled in a small number of instances by subsequent post-mortem examination [7]

It may, therefore, be stated that the electrocardiographic curves obtained as a result of cardiac hypertrophy, from whatever cause, depend essentially on the degree and distribution of the hypertrophy present in a given case, whether left or right side predominates

In the presence of left ventricular preponderance the resulting electrocardiogram is characterized by a tall, conspicuous initial deflection R in Lead I, with an inconspicuous S, and by a deep deflection S, with an inconspicuous R, in Lead III Lead II is less characteristic

In the presence of right ventricular preponderance the picture is reversed In Lead I, R is inconspicuous, the initial deflection S appearing as a deep depression, while in Lead III, R appears as a tall conspicuous peak, and S is small

7 Lewis Clinical Electrocardiography, London, 1913, p 24

It is apparent that, as in the aberrant electrocardiogram, the most prominent initial deflections in Leads I and III are in opposite directions, the direction depending on the predominance of one or the other ventricle The electrocardiogram obtained from a case of simple cardiac hypertrophy may be distinguished from the aberrant type, which it resembles in many respects, by the shorter duration of the opening phases of the complex, by the lesser amplitude of the prominent deflections, by the direction of the deflection T in relation to the deflections R and S, by the absence of notching of R and S, and by the normal triphasic or quadriphasic character of the complex In aberrant curves T' is in the opposite direction to the main initial deflection (R' or S') in Leads I and III, in the curves of hypertrophy T and R or S are often in the same direction in these leads

When, as occasionally happens, a temporary defect in conductivity occurs in the course of an acute breakdown of an already hypertrophied heart, a condition almost always present in these cases, one may be able to compare the aberrant curve obtained from such a heart with the curve of simple hypertrophy obtained later, after the disappearance of the transient functional defect

Such instances are not common, but the occurrence of a transient aberrant electrocardiogram is most important from the point of view of a comparison of the aberrant curve with the physiological type in the same case

Allusion has been made to the single instance of such temporary defect in conductivity included in this series, and the facts stated above are well illustrated by a comparison of Figures 8 and 9, which accompany Case 12, the bizarre aberrant electrocardiogram resulting from the acute lesion of the right branch of the bundle, giving place, on the subsidence of the acute process, to the physiological electrocardiogram recorded on the following day

No change could be more marked the earlier curve due to defective conduction plus left ventricular preponderance, the latter essentially the curve of simple cardiac hypertrophy No more striking evidence than these two figures could be offered that the aberrant electrocardiogram does not depend on the presence of cardiac hypertrophy

In this connection reference may also be made to Case 20, Figures 16 and 17 Here we are fortunate in having obtained the physiological electrocardiogram of the individual, before the development of defective conductivity, for comparison with the curves obtained later and after the development of the lesion involving the right branch The difference between the two figures is apparent at a glance the earlier physiological electrocardiogram could not by any possible chance be confused with the aberrant complex obtained later, after the development of defective conductivity

SIDE OF THE DEFECT

In the twenty-two cases of this series the right branch of the auriculoventricular bundle has been involved in twenty-one, the left branch in but one instance The majority of previously described cases also are those in which a defect of the right branch has been diagnosed

TABLE 2.—SUMMARY OF CARDIOGRAMS IN CASES 1 TO 22 *

Patient	Case No	Fig No	*P R* Interval Seconds
D B	1		0 13
G S	2		0 24
J P	3		A F
H C	4	1	0 20
J P	5	2	A F
R S	6		0 34 (1-17-11)
			0 30 (8 7-12)
			C H B (11-3-12)
			Dropped beats (11-13-12)
		3	Dropped beats (2-6 13)
			C H B (11 24 13)
W S	7	4	0 21 (three observations)
H C	8		0 29
S R	9	5	0 17-0 18
R B	10	6	0 21-0 23 (four observations)
C H	11		0 26 (6 12-12)
			0 16 (7-12-12)
		7	0 20 (7-25-12)
W C	12	8	0 18 (8 18 12)
		9	0 18 (8-19 12)
			0 20 (10 6 12)
R A	13	10	0 13
W P	14		A F
J W	15	11	0 20
D E	16	12	0 14
W O	17		0 28 (2 17-13)
		13	0 17 (5 12 13)
G C	18	14	0 20
W J	19	15	0 18 0 20 (two observations)
D H	20	16	0 30 (2-27-13)
			0 24 (3 5-13)
			0 24 (4 7-13)
			0 21 (4-10-13)
			0 24 (4 28 13)
		17	0 22 (5 5 13)
			0 29 (5 15 13)
J M	21	18	0 17
E N	22	19	C H B

* The conditions found in Cases 1 to 22 are as follows Aortic disease, 7, 9, 10, 11, 17, 18, 20, 21, varying degrees of heart block, 2, 4, 6, 7, 8, 10, 11, 12, 15, 17, 18, 20, 22, Cheyne Stokes respiration, 2, 3, 5, 16, auricular fibrillation, 3, 5, 14, various other associated pathological states, 1 4, 8, 13, 15, 19, 22 Subsequent histories are Dead, Patients 2, 3, 5, 7, 9, 10, 11, 14, 16, 18, 19, 20, alive (condition precarious) 8, 12, 13, 17, 21, 22, alive (condition not precarious) 1 and 4, history unknown (condition precarious when last seen) 6 and 15

There is no entirely satisfactory explanation of this difference in incidence It is possible, however, that the predominance of the right-side lesion may be explained on anatomical grounds, the right branch, as a circumscribed track, being by far the longer.

Table 2 summarizes the cases reported in detail In the column giving the *P-R* intervals, these have been grouped in chronological order, otherwise, so far as is possible, wholly on the basis of the associated pathological condition

RELATION TO AURICULOVENTRICULAR BLOCK

Deficiency in the conduction of the branches of the bundle has a close relation to a similar deficiency in the main stem of the auriculoventricular trunk It has been emphasized by several writers, Sternberg[8] and Monckeberg[9] among others, that the conducting tissues which unite the auricles and ventricles have a more or less special pathology Aschoff[10] has called attention to their liability to damage from small hemorrhages, as a result of their curious sheathing with connective tissue; and it is known that fatty degeneration falls unequally on the conducting tissues and the rest of the muscle of auricle and ventricle Aschoff[11] and Butterfield[12] have shown that the infiltration in rheumatic carditis is observed in the subendocardial tissues and that it is especially prone to invade the conjunctional tissues. That the conducting tissues have a special physiology is certain, for their glycogen content is known to be much greater than that of ordinary cardiac muscle (Nagayo[13])

Dealing with the same question from a purely clinical point of view the facts point clearly in the same direction. The normal human *P-R* interval usually varies between 0 13 and 0 18 seconds, a higher value than 0 18 is very exceptional, the commonest values are from 0 13 to 0 16 seconds, as shown by Lewis and Gilder[14] In the present series of twenty-two cases, a sequential rhythm has been present in eighteen In thirteen of these eighteen cases the *P-R* interval was prolonged, constantly or on occasion In four (Cases 6, 12, 17 and 20) it showed considerable variation One of these (Case 6) showed auriculoventricular heart-block of extraordinarily varying degree, namely, simple prolongation of the interval, dropped beats and complete dissociation In another of the series (Case 22) complete dissociation was a constant feature

8 Sternberg Verhandl d deutsch path Gesellsch, 1910, xiv, 102
9 Monckeberg· Verhandl d deutsch path Gesellsch, 1912, xvi, 460
10 Aschoff Verhandl d deutsch path Gesellsch, 1910, xiv, 3
11 Aschoff Brit Med Jour, 1906, ii, 1103
12 Butterfield Heart, 1912, iii, 203
13 Nagayo Verhandl d deutsch path Gesellsch, 1908, xii, 150
14 Lewis and Gilder Phil Tr Roy Soc, 1912, ccii, 351

This clinical evidence speaks very strongly for the frequency of functional damage of the conducting tissues as a whole, though necessarily the damage varies in extent in different parts of the system from case to case In many of the published cases of complete heart-block, trunk-branch lesions are demonstrated by the electrocardiographic curves [15]

Experimental evidence points in the same direction, Lewis and Mathison[16] have shown that asphyxia of cats produces auriculoventricular heart-block, and that at the same time it frequently damages the conduction in the main branches of the auriculoventricular system

We have every reason to conclude, from the evidence derived from all sources, that inflammatory lesions and invasions of the heart by poisons may act in a more or less specific manner, picking out the special system of muscle fibers which unite the auricle and ventricle The physiology and the pathology of these tissues is largely a special physiology and pathology

RELATION TO AORTIC VALVE DISEASE

It is a remarkable fact that out of the present series of twenty-two cases, eight patients have exhibited well-developed aortic valve lesions The frequent association of damage of the branches of the auriculoventricular bundle with aortic valve disease can not be regarded as purely accidental Yet the association meets with no very plausible explanation, especially so because in each of the eight cases the right trunk was defective Lesions of the aortic valve would be expected, *a priori,* in association with lesions involving the conductivity of the left branch, for the bases of the valves are in close proximity to it Nevertheless this association does not occur Too much emphasis may be laid on this argument, it is weakened by a knowledge of the relative frequency of lesions of the right and left trunk-branches, the former are far commoner In the present series the right branch was defective in twenty-one cases, the left in one only

An explanation that the curves are in reality but an expression of left-side muscular preponderance does not suffice It is perfectly true that the curves of the aberrant type, associated with right-branch lesions, and those speaking for left-side preponderance resemble each other, but there are, nevertheless, clear and recognizable distinctions between them, distinctions which have been emphasized already and which are to be seen in the curves of Cases 12 and 20

15 See the case reported by Cohn and Lewis, already referred to, and the second case of Mathewson's series, to which reference has been made

16 Lewis and Mathison Heart, 1910, II, 47

We are able to produce very satisfactory evidence that the curves in question are not those of left-side preponderance pure and simple, for in these two instances the physiological electrocardiograms for the particular individuals have been obtained for comparison In Case 12, the change in the type of electrocardiogram is profound and sudden, the aberrant type of curve (Fig 8) could not result purely from hypertrophy, when within a day an entirely different and more normal type of curve is displayed (Fig 9) The reason for the frequent association of aberrant curves with aortic valve disease is, therefore, still obscure

RELATION TO EXTRASYSTOLES

The common occurrence of extrasystoles in conjunction with aberrant forms of electrocardiograms suggests that the lesion which produces the conduction defect may also awaken the new impulses, this the more, since it has been suggested that extrasystoles are often of bundle or bundle-branch origin It is necessary to examine the direction of the deflections from this point of view Our curves give us only meager information Though extrasystoles were seen in a much larger proportion of the cases, the curves in our possession show them in seven instances only We consider Leads I and III as of most importance from our present point of view The aberrant curves are mainly diphasic, the curves due to extrasystoles are also mainly diphasic, but in a given case the deflections of the diphasic complexes are usually in opposite directions when aberrant and extrasystolic curves are compared (Table II)

That is to say, in most cases the extrasystole arises in the ventricle opposite to that in which the aberrant beat originates This is what would be expected if a lesion produced defective conduction in the branch, and also provoked extrasystoles from it Thus in Cases 10, 14 and 22 the extrasystoles arose from the right or basal portion of the heart, as shown by all leads In Case 3 the extrasystoles were, on the other hand, from the left and apical portion of the ventricle In the remaining Cases 11, 15, 20, the deflections of the extrasystole were opposite to those of the aberrant curve in one lead (Lead II), but in the same direction in another lead (Lead I or Lead III)

RELATION TO PROGNOSIS

The prognostic value of any given sign in disease of the heart is always a difficult problem Especially is this the case when the sign is frequently associated with other signs of a grave nature

An organic lesion confined to one branch of the auriculoventricular bundle is hardly one which can be regarded as jeopardizing life We

find that a bundle-branch lesion may be present for many years and the heart still show general efficiency (Cases 1 and 4) But just as lesions of the branches are rarely confined to the branches, but are associated with defects in the main bundle, so too the defects are probably but rarely confined to the conducting system, but spread beyond it into the general musculature to a lesser or greater extent

From analogy with auriculoventricular heart-block, we must regard a bundle-branch lesion as significant of an invasion of the heart-muscle, be it acute or chronic, and therefore of considerable gravity in most instances In the present series of cases, the gravity of such a lesion is well illustrated In twenty-two cases, reports of which have been

TABLE 3.—SUMMARY OF CARDIOGRAMS IN CASES 1 TO 22 SHOWING OCCURRENCE OF PREMATURE BEATS *

Case No	Direction in Relation to Initial Deflections	Lead
3	Same	I, III
10	Opposite	I, II, III
11	Opposite	II
11	Same	III
14	Opposite	I, II, III
15	Same	III
20	Opposite	II
20	Same	I
22	Opposite	I, III

* In cases 2, 16, 17, 21, premature beats were present, but were not recorded electrocardiographically

collected within a few years, twelve patients are known to have died (Patients 2, 3, 5, 7, 9, 10, 11, 14, 16, 18, 19, 20) Six are alive but known to be in a precarious condition (Patients 8, 12, 13, 17, 21, 22), two have been lost sight of, but were in a precarious condition when last seen (Patients 6 and 15) Two only (Patients 1 and 4) have been under observation for considerable periods and show no signs of increasing heart-failure

The frequent association of the condition with alternation of the heart, auricular fibrillation (Cases 3, 5, 14), Cheyne-Stokes respiration (Cases 2, 3, 5, 16), great enlargement of the heart and signs of circulatory embarrassment is in itself significant

REPORT OF CASES

CASE 1.—*Myocarditis, fibrosis of the left lung with bronchiectasis*

Patient.—D B, a girl, aged 16, was admitted to the hospital during the year 1906, complaining of cough, shortness of breath and pain in the left side of the chest

Family History.—A sister has had rheumatic fever Her father died of pneumonia complicated by "heart disease"

Personal History—A delicate child at birth, the patient has had a cough all her life, though it has become much more troublesome of late She has had no acute illnesses There is no history of rheumatic fever or chorea The shortness of breath, especially prominent on exertion, and the pain in the chest are recent developments She brings up a little yellow sputum

Condition on Admission—The patient is poorly developed, with a certain degree of scoliosis, the curvature being toward the left side The color of the cheeks is heightened There is impairment of resonance over the whole left side of the chest both in front and behind, the capacity of this side is diminished In the left intrascapular region the breath sounds are tubular and bronchophony and pectoriloquy are heard, coarse clicking râles are audible over the same region, some of which synchronize with the heart-beats A little purulent sputum contained no tubercle bacilli The respirations are normal

Heart—Pulsation is seen over almost the whole left chest in front The point of maximum impulse is diffuse in the sixth interspace near the posterior axillary line, posteriorly it is thrusting in character A diastolic thrill is felt over the apex region The cardiac dulness extends from the third rib above, and from the left margin of the sternum to the posterior axillary line Systolic and diastolic murmurs are heard at the apex A harsh systolic murmur is present at the aortic cartilage The pulse is 76 to the minute and regular, the arteries are soft There are no signs of venous engorgement or of dropsy The urine contains no albumin

Condition on Readmission (Nov 9, 1912)—After six years the patient has been readmitted for the same symptoms Pain has been present for twelve months, and shortness of breath, which has never entirely abated, is more distressing From time to time she brings up large quantities of sputum The physical signs at this time are very similar to those previously observed The signs over the fibrosed lung are almost unaltered The limits of cardiac dulness are the same The murmurs remain unchanged The respirations are not increased, there is but little venous engorgement, dropsy is absent, the urine contains no albumin The temperature never rises above 99 F

Electrocardiograms—The P-R interval measured 0 13 second In Leads I and II the initial deflection was abrupt and notched, having a total duration of 0 1 second In Lead III an upward phase was succeeded by a larger downward phase, the two together measuring 0 1 second

Subsequent History (June 21, 1913)—The patient is alive and in fair condition

CASE 2—*Myocarditis, extrasystoles, alternation, Cheyne Stokes breathing and death*

Patient—G S, a business man, aged 63, was first seen April 27, 1910, complaining of cough and shortness of breath on exertion

Personal History—With the exception of occasional attacks of gout the patient has had no illness since his boyhood He has complained of his present symptoms for the past five months, these consist chiefly of an irritating cough, which is accompanied by considerable shortness of breath

Condition on Admission—The patient is well developed and shows no sign of cyanosis He has Cheyne-Stokes breathing

Heart—The cardiac dulness extends two inches to the left of the nipple line The first sound at the apex is reduplicated There are no murmurs The pulse is full and intermits from time to time The blood-pressure is 125 mm Hg

Graphic Records—Polygraphic records showed that the intermissions of the pulse were the result of premature contractions of the ventricle These are followed by alternation The *a-c* interval is distinctly prolonged

Subsequent History—The patient died twelve months later

Electrocardiograms—The P-R interval measured 0.24 second. The ventricular curves were similar to those shown in Figure 8.

CASE 3.—*Rheumatic myocarditis, high blood-pressure, auricular fibrillation, alternation, Cheyne-Stokes breathing, nephritis, urethral stricture, gradual failure and death.*

Patient.—J. P., a shop-keeper, aged 65, was admitted to the hospital Aug. 1, 1911, complaining of shortness of breath, pain in the chest, loss in weight and sleeplessness.

Personal History.—At the age of 26, 32 and 48 the patient had rheumatic fever. At 59 he had a slight "apoplectic stroke" and lost the sight of his left eye. He has been treated for urethral stricture for eighteen years.

Present Illness.—The patient states that twelve months ago he was quite free from any discomfort. About this time he began to suffer from shortness of breath, which has steadily increased, at present the slightest exertion brings it on. He has experienced shooting pains in the left side of the chest, and also, from time to time, in the right hypochondrium. The breathing is said to be heavy and periodic at night. He has lost 42 pounds in weight. He sleeps but two or three hours each night. His appetite is poor.

Condition on Admission.—The patient is well nourished and cyanotic and exhibits conspicuous Cheyne-Stokes breathing. The liver is palpable and tender an inch below the costal margin. There is slight edema of the legs and some ascites present. The specific gravity of the urine ranges from 1.010 to 1.016, albumin, sugar and pus are present in excess. The pulse is very irregular, the rate being 90 to 120 per minute, the arteries seem but little thickened. Most of the pulse beats pass a cuff-pressure of 200 mm. Hg.

Heart.—The apex-beat is not visible, the limits of cardiac dulness lie 2 inches to the right and 5 to the left of the midsternal line. The heart sounds are clear, but very irregular, there are no murmurs.

Course of Disease.—The patient remained in the hospital for some weeks and improved a good deal in health. He passed from observation, but his wife wrote subsequently to say that he gradually became weaker, thinner and more short of breath. Dec. 5, 1911, when sitting up in a chair, he asked to be put to bed again, but almost immediately he became relaxed and died.

Electrocardiograms.—The P-R interval could not be determined, owing to the constant presence of auricular fibrillation. The ventricular complexes were similar to those shown in Figure 6. The extraventricular systoles recorded arose from the left and apical portion of the heart, being in the same direction as the initial deflections *R'* and *S'* in Leads I and III.

CASE 4.—*Pericarditis with effusion, arhythmia, drainage of the pericardium and recovery* (Electrocardiograms, Fig. 1.)

Patient.—H. C., a woodcarver, aged 28, was admitted to the hospital Aug. 8, 1911, complaing of indigestion and palpitation of the heart.

Family History.—Negative.

Personal History.—The patient had pneumonia as a child and so-called "muscular rheumatism" two years ago. There is no history of rheumatic fever or chorea. He denies syphilis.

Present Illness.—For the past eight weeks the patient has complained of indigestion and palpitation of the heart. Four weeks ago, July 12, he had a severe attack of vomiting, and has had frequent similar attacks since this time. Since the onset of these attacks of vomiting he has been quite breathless on slight exertion, and latterly has noticed that there was some swelling of his legs.

Condition on Admission.—Edema of the legs and scrotum and well-marked signs of compression of the left lung are present. The liver is palpable at the

umbilicus The temperature is 99 8 F The respirations are from 28 to 32 per minute

Heart—The upper limit of cardiac dulness lies at the first interspace, to the right 3¾ inches and to the left 8 inches from the midsternal line Pulsation over the precordium is very slight There is a distant soft systolic murmur heard over the region of the apex The first and second sounds are heard everywhere, but are very distant There is a pleuropericardial friction-rub heard in the third and fourth left interspaces

Course of Disease—Aug 13, 1911 The fourth left costal cartilage was removed, the pericardium opened and a pint of hemorrhagic fluid withdrawn

Aug 18, 1911 For several days the patient has had irregularity of the heart, the result of fibrillation of the auricles To day the pulse is regular, the *a* wave has appeared again

Aug 25, 1911 The cardiac dulness has been greatly reduced by tapping

Sept 14, 1911 The patient was tapped again to day and 2½ pints of blood-stained fluid were removed

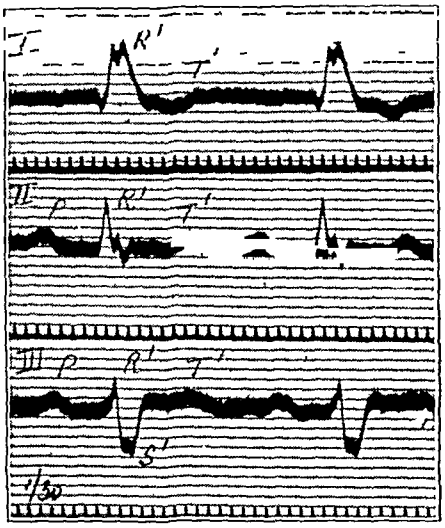


Fig 1—Electrocardiograms Case 4 The *P R* interval measures 0 2 second The *Q R S* interval is increased, measuring 0 13 second In Lead I, the initial deflection *R'* is conspicuous as a broad summit with a markedly split apex The final deflection *T'* is inverted, lying in a direction opposite to the prominent summit *R'* In Lead II, the initial deflection *R'* appears as a conspicuous peak with marked notching The final deflection *T'* is upright, lying in the same direction as *R'* In Lead III, *R'* is present, but *S'* appears as a prominent broad depression with marked notching of the base The final deflection *T'* is upright, lying in a direction opposite to the prominent deflection *S'* The increased duration of the opening phases of the complex, the conspicuous notching, and the direction of the final deflection *T'* in relation to prominent deflections *R'* and *S'*, are apparent

Sept 26, 1911 A note was made as follows The pericardium is filling again The pulse has remained almost continuously regular On one or two occasions the patient has noticed some palpitation of the heart The temperature has been normal throughout, except for a few days after the operation

Sept 11, 1912 The patient was seen on this date and is apparently in very fair condition There is very little breathlessness The limits of cardiac dulness are considerably reduced and no effusion is demonstrable in the pericardium

Subsequent History—July, 1913 The patient is alive and apparently in good condition

CASE 5—*Cardiac hypertrophy, myocarditis, auricular fibrillation, arteriosclerosis, Cheyne-Stokes respiration, cardiac failure and death* (Electrocardiograms, Fig 2)

Patient—J P, a hotel keeper, aged 69, was admitted to the hospital Nov 11, 1911, complaining of breathlessness, feelings of faintness and swelling of the legs

Family History—Negative

Personal History—The patient remembers no past illness He has not had rheumatic fever and denies syphilis He has drunk beer in moderation, but has been a heavy smoker

Present Illness—In October, 1907, the patient met with an accident, which he states injured his spine He was laid up at this time some weeks, but made a good recovery, though he insists that his heart has been weak since the mishap In October, 1911, he contracted a severe cold and had a slight pneumonia, being ill in bed for some weeks From this time his symptoms have increased At present he complains of great breathlessness on slight exertion, general lassitude and frequent attacks of dizziness Recently his legs have become swollen and he has noticed puffiness about the eyes

Condition on Admission—The patient is well built, but exhibits conspicuous breathlessness There is a slight degree of cyanosis present The legs are edematous, the liver is not palpable, the flow of urine is abundant and albumin is excessive The pulse is very irregular and weak (auricular fibrillation), rate 80 per minute The arteries show thickening, the systolic blood pressure is 160 mm Hg (strongest beats)

Heart—The heart's impulse is not visible, the limits of dulness are difficult to ascertain as the lungs encroach so much on it The heart sounds are faint, the first sound is reduplicated, no murmurs are heard

Course of Disease—During the patient's stay in the hospital Cheyne Stokes breathing was often observed, he became delirious from time to time, and on occasions was in a state of coma He was treated with digitalis and, though his life was despaired of, he was eventually discharged a good deal improved

Readmission—A year later he was again admitted with the same symptoms Nov 1, 1912 The legs are again swollen, he has much cough and breathlessness and sleeps badly His symptoms have been increasing for the past three weeks and he has lost in weight The physical signs are much the same as on his first admission

The heart is still irregular, but of normal rate The upper limit of cardiac dulness lies at the third interspace, to the right 1½ inches and to the left 5½ inches from the midsternal line There are no murmurs, the first sound is faint, but not reduplicated The liver is just palpable

Course of Disease—Cheyne Stokes respiration was observed on many occasions and he was sometimes delirious at night Although constantly breathless cyanosis was never prominent He again so far recovered as to be able to leave the hospital much improved

Readmission (March 3, 1913) (Note T L) The patient lies propped up in bed His lips are pale and very slightly cyanotic His breathing is labored and from time to time shows periodicity in depth There is slight edema of the ankles The pulse rate is 84 to the minute and irregular (auricular fibrillation and defect of the right bundle branch as before) The signs in the heart remain unaltered There is impairment of resonance at the bases of both lungs The liver is easily palpable below the costal margin

March 5, 1913 The respirations are irregular and show periodicity in depth No apneic periods, however, are present

March 19, 1913 There is to day a good deal of improvement in the patient's breathing, it is easy and almost natural during the day, but tends to show a periodicity at night

March 21, 1913 The patient was discharged to day in a much improved condition There was at this time no breathlessness if he moved slowly, and his

color was good During his stay in the hospital the tempera
The systolic blood-pressure ranged between 130 and 140 mm
varied in quantity between 1,100 and 2,500 c c per diem
acid, the specific gravity ranging from 1 010 to 1 012, except
sion, when it was 1 022, albumin, blood and casts were not f

Readmission (April 3, 1913) —Seen in the out-patient's de
day, the patient was evidently much worse and was readmitte
He has been voiding very little urine and what is passed is d
is considerably, but not deeply, cyanosed and is very dyspnei
is rapid, 32 to the minute, and labored, while he lies well
liver is again large, and there is impairment of resonance at
lungs and numerous crepitations are heard over the whole che
the cardiovascular system are unaltered

April 6, 1913 Cheyne Stokes breathing with long apneic p
at night Patient is still cyanotic Electrocardiograms we
date

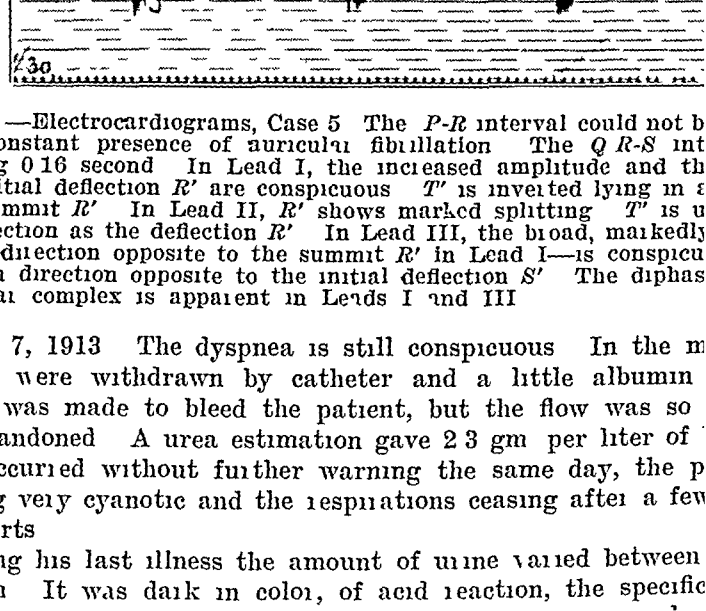


Fig 2—Electrocardiograms, Case 5 The *P-R* interval could not b
to the constant presence of auricular fibrillation The *Q R-S* int
measuring 0 16 second In Lead I, the increased amplitude and th
of the initial deflection *R'* are conspicuous *T''* is inverted lying in a
to the summit *R'* In Lead II, *R'* shows marked splitting *T''* is u
same direction as the deflection *R'* In Lead III, the broad, markedly
S'—in a direction opposite to the summit *R'* in Lead I—is conspicu
lying in a direction opposite to the initial deflection *S'* The diphas
ventricular complex is apparent in Leads I and III

April 7, 1913 The dyspnea is still conspicuous In the m
of urine were withdrawn by catheter and a little albumin
attempt was made to bleed the patient, but the flow was so
to be abandoned A urea estimation gave 2 3 gm per liter of
Death occurred without further warning the same day, the p
becoming very cyanotic and the respirations ceasing after a few
tory efforts

During his last illness the amount of urine varied between
per diem It was dark in color, of acid reaction, the specific

Post-Mortem Examination (April 8, 1913).—Deep cyanosis. Dropsy of the legs. The pleural cavities each contain about one pint of clear fluid. There is an excess of fluid in the pericardial and peritoneal cavities. The lungs are slightly emphysematous, the bases are edematous and congested. The heart is large and covered with fat, the muscle shows yellow striation. The cavities are dilated and the walls seem hypertrophied. The valves are normal. No ante mortem clots were found. The aorta and large arteries show generalized arterio sclerosis with calcareous deposits, the aortic arch is dilated. The liver is large, fatty and congested. The splenic pulp is friable and dark in color. The kidneys are congested, the capsules strip off leaving a slightly torn and granular surface, there are a few scars on the surface, but no cysts. The cortex and medullary substance show narrowing. The brain is normal though the membranes over the vertex are edematous, the remaining organs are normal.

Electrocardiographic curves taken on repeated occasions always showed the same mechanism, namely, fibrillation of the auricles and absent conduction in the right branch of the auriculoventricular bundle. The ventricular rate was always slow, ranging from 60 to 90. (See Fig. 2.)

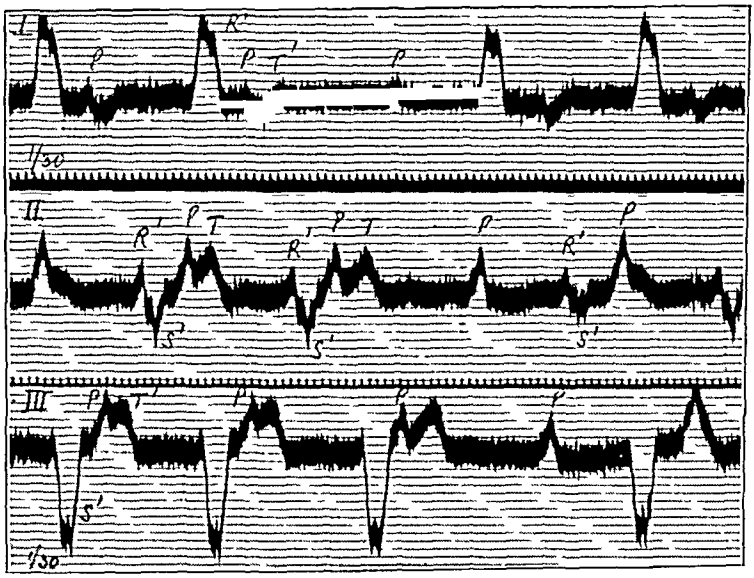


Fig. 3.—Electrocardiograms, Case 6. Note the degree of heart block present. From time to time a failure of response to the previous auricular contraction is shown. In Lead I, the initial deflection *R'* appears as a conspicuous summit of increased amplitude and duration, with marked notching. The final deflection *T'* is inverted, lying in a direction opposite to the prominent *R'*. The gradual increase in the *P R* interval up to the point of the occurrence of the dropped beat is apparent. There is no ventricular response to the second *P* summit in this lead. In Lead II, *R'* is present but the deflection *S'* appears as a conspicuous depression of increased amplitude and duration with marked notching. The final deflection *T'* is upright, lying in a direction opposite to the prominent *S'*. Here again the failure of a ventricular response, following the second auricular contraction, is apparent. In Lead III, *R'* is absent. The initial deflection *S'* appears as a depression of greatly increased amplitude and duration, with conspicuous notching. The final deflection *T'* is upright, lying in a direction opposite to the prominent *S'*. There is a missed ventricular response after the third *P* summit.

CASE 6.—*Cardiac hypertrophy, mitral disease, paroxysmal tachycardia and auriculoventricular heart block in varying degrees* (Electrocardiograms, Fig. 3.)

Patient.—R. S., a bootmaker, aged 64, was admitted to the hospital Dec. 1, 1911, complaining of shortness of breath, attacks of faintness and pain in the chest.

Personal History.—Twelve years ago the patient suffered from a fistula *in ano*. There is no history of any other illness. He denies syphilis. He has been a moderate user of alcohol and tobacco.

Present Illness—Twelve months ago he first noticed shortness of breath on exertion For six weeks he has experienced attacks of pain in the chest accompanied by faintness In some of these attacks he vomits, and on one occasion he lost consciousness His pulse rate in the attacks, which were witnessed by his physician, Dr Alex Brown of Kentish Town, rose to at least 250 per minute

Condition on Admission—The patient is well nourished and shows no sign of breathlessness and little or no cyanosis The liver is enlarged There is no dropsy The temperature is normal The pulse is regular, its rate being from 72 to 144 per minute, the vessel-wall is thickened The systolic blood-pressure varies between 64 and 130 mm Hg

Heart—The heart's impulse is neither visible nor palpable The limits of cardiac dulness lie at the fourth rib above, to the right at the left sternal margin, and to the left in the fifth space four inches from the midsternal line There is a blowing systolic murmur at the apex, and also a systolic murmur at the aortic cartilage

Subsequent History—Unknown

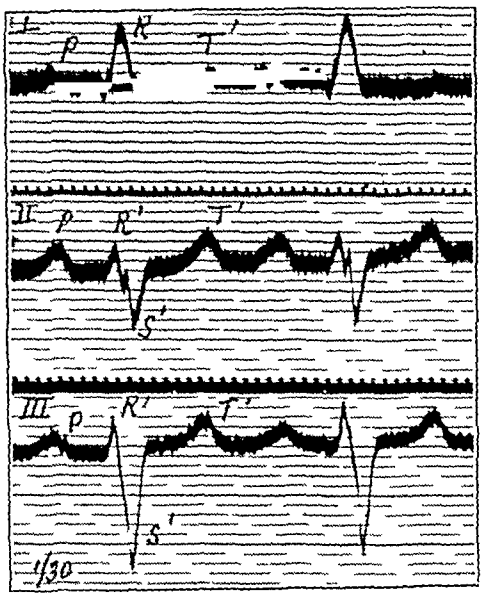


Fig 4—Electrocardiograms, Case 7 The *P R* interval measures 0 21 second The *Q-R S* interval measures 0 11 second In Lead I, the initial deflection *R'* appears as a peak of increased duration and slight increase in amplitude, with conspicuous notching The final deflection *T''* is upright, lying in the same direction as the initial *R'* In Lead II, *R'* is present but inconspicuous The deflection *S'* appears as a depression of increased duration with marked notching The final deflection *T''* is upright, lying in a direction opposite to *S'* In Lead III, the initial deflection *R'* is again inconspicuous The deflection *S'* appears as broad depression of greatly increased amplitude The final deflection *T''* is upright, lying in a direction opposite to the prominent *S'* The increase in the *P R* interval is apparent in all three leads

Electrocardiograms—This case is of especial interest because of the presence of varying degrees of auriculoventricular heart block, which were present at different times, the patient having been under observation over a long period before his entrance into the hospital (Fig 3) Jan 17 1911, the *P-R* interval measured 0 34 second, and Aug 7, 1912, 0 3 second Nov 3, 1912, complete auriculoventricular heart block was present Nov 13, 1911, dropped beats, Feb 6, 1913, dropped beats, and again Feb 24, 1913, complete auriculoventricular heart-block was present

CASE 7—*Arteriosclerosis, cardiac hypertrophy, aortic disease, myocarditis, cardiac failure and death* (Electrocardiograms, Fig 4)

Patient—W S, a coal porter, aged 52, was admitted to the hospital Feb 24, 1912, complaining of abdominal pain, shortness of breath and sleeplessness

Family History—Negative

Personal History—The patient gives no history of any past illness Syphilis and rheumatic fever are both denied For many years he has drunk six pints of beer daily and has used four ounces of tobacco a week His work has been heavy

Present Illness—The patient states that his present illness began about a month ago (Jan 24, 1912) At this time he first noticed shortness of breath on any slight exertion This was soon followed by dull aching pains referred to the upper abdomen, and by sleeplessness The shortness of breath has gradually increased, a troublesome cough has developed and latterly he has complained of feeling sick on rising in the morning

Condition on Admission—The patient is pale and orthopneic There is no cyanosis The teeth are carious The temperature is normal There is neither dropsy nor ascites The respirations are 36 to the minute The urine is normal The pulse is regular, 90 to the minute, of the water-hammer type Capillary pulsation is present Pulsation of the carotids is visible to the lobes of the ear The arteries are thickened The systolic blood pressure is 110 140 mm Hg The liver is palpable an inch below the costal margin

Heart—The point of maximum impulse is in the fifth and sixth interspaces five inches from the midsternal line The upper limit of cardiac dulness lies at the third rib, to the right one inch and to the left five inches from the midsternal line At the apex and over the aortic area systolic and early diastolic murmurs are heard, the first sound is inaudible

Subsequent History—During a month's stay in the hospital his acute symptoms, with the exception of slight breathlessness on exertion, entirely subsided The respirations fell to 20 per minute when at rest Otherwise his condition remains unchanged He left the hospital but died some time afterward, no necropsy was obtainable

CASE 8—*Cardiac hypertrophy, myocarditis, mitral disease and pericarditis*

Patient—H C, a domestic, aged 18, was seen March 16, 1912, complaining of shortness of breath and some pain in the chest, in front A note made at this time states that the patient had pneumonia at the age of 5, she is also said to have had swelling of the knees and ankles on several occasions, which suggest a rheumatic history There has been at different times some edema of the legs Infantilism is conspicuous

Heart—The limit of cardiac dulness lies 3½ inches to the right and 6 inches to the left of the midsternal line There is an early diastolic and systolic thrill at the apex The first sound at the apex is reduplicated, associated with early diastolic murmurs A friction sound is heard over the base of the heart The aortic and pulmonary sounds are normal

Subsequent History (June 14, 1913)—The patient is alive, but her condition is much as noted above

Electrocardiograms—The *P-R* interval measures 0 29 second In Lead I, *S'* and *T'* deflections are seen, the whole curve being diphasic and the initial phase (*S'*) long In Lead II the curve is similar, but a small spike *R'* is added In Lead III, *R'* opens the complex and is followed by a *T'* deflection, a small *S'* is also present

CASE 9—*Myocarditis aortic regurgitation, cardiac failure and death*

Patient (Electrocardiograms, Fig 5)—S R, a gas fitter, aged 36, was admitted to the hospital April 9, 1912, complaining of shortness of breath, cough, swelling of the feet and sleeplessness

Personal History—There is no history of any past illness The patient has seen service in South Africa

Present Illness—The onset of his present illness is attributed by the patient to an attack of sore throat and bronchitis, which he had four months ago (January, 1912) He was examined at this time and his heart was found to be

affected Recently the shortness of breath has increased to such an extent that he cannot take the slightest exercise and work is impossible

Condition on Admission—The patient is pale but well nourished There is slight cyanosis The respirations are hurried, 28 to the minute, a little labored Some venous engorgement and signs of pulmonary congestion are present There is edema of the feet and back The specific gravity of the urine is 1 023, reaction acid, no albumin is present The liver is palpable three finger-breadths below the costal margin The pulse is regular, 106 to the minute, of the water-hammer type Capillary pulsation is present, though slight The arteries are tortuous

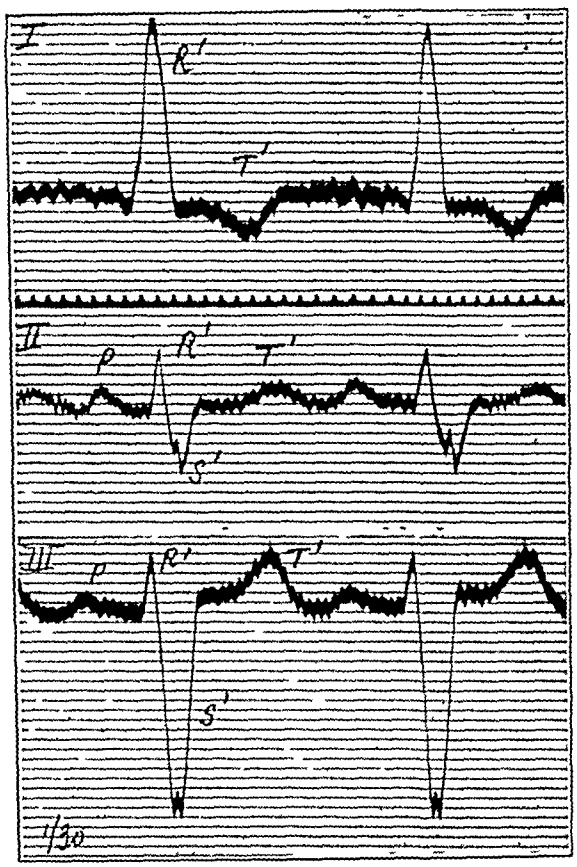


Fig 5—Electrocardiograms, Case 9 The *P-R* interval measures 0 17 0 18 second The *Q R S* interval measures 0 1 second to 0 11 second In Lead I, the initial deflection *R'* is conspicuous as a tall broad summit with slight notching *T''* is inverted lying in the opposite direction to the summit *R'* In Lead II, the initial deflections *R'* and *S'* are equally conspicuous *S'*, however, shows marked notching *T''* is upright in a direction opposite to the deflection *S'* In Lead III, the deflection *R'* is present as a short summit, while the depression *S'* is conspicuous as a deep broad deflection with split base *T''* is upright in a direction opposite to the prominent deflection *S'* The greatly increased amplitude, the longer duration and the notching of the initial deflections *R'* and *S'* are conspicuous in all leads

Heart—The shock of the heart is diffuse and extends well outside the nipple line The limit of cardiac dulness lies 2 inches to the right and 6¼ inches to the left of the midsternal line At the apex a systolic and a faint diastolic murmur are heard, over the aortic area to-and-fro murmurs are heard, maximal in intensity at this point, over the pulmonary area the same murmurs, though fainter, are audible The second sounds are not heard

Course—After a month's stay in the hospital the patient's condition improved considerably, and he was discharged at his own request A few weeks later he was admitted to an infirmary, with an exacerbation of all his symptoms and with aggravated dropsy He died within a few hours No necropsy was obtained

CASE 10.—*Rheumatic carditis, aortic disease, angina pectoris, high blood-pressure, alternation, cardiac failure and death* (Electrocardiograms, Fig 6)

Patient.—R. B., aged 45, came under observation June 6, 1912, complaining of pains in the chest and breathlessness.

Family History.—The patient's father and an uncle died suddenly of "heart disease." A sister has had rheumatic fever. The patient's child, aged 9, was examined and found to have mitral stenosis.

Personal History.—The patient has suffered from rheumatic fever on five occasions, the first attack being at the age of 12 and the last at 30. He became almost deaf at 40. At 43 he had an attack of pleurisy. Syphilis is denied.

Present Illness.—The present illness really began twenty eight years ago, when the patient was treated at a London hospital for angina pectoris. The pains have been severe of late and radiate over a wide area, they are accompanied by feelings of giddiness, suffocation and anxiety, and are brought on by the slightest exertion. Lately his legs have become swollen and breathlessness has developed.

Condition on Admission.—The patient is pale and poorly nourished. There is a little dropsy of the extremities. Cyanosis and dyspnea are absent. The

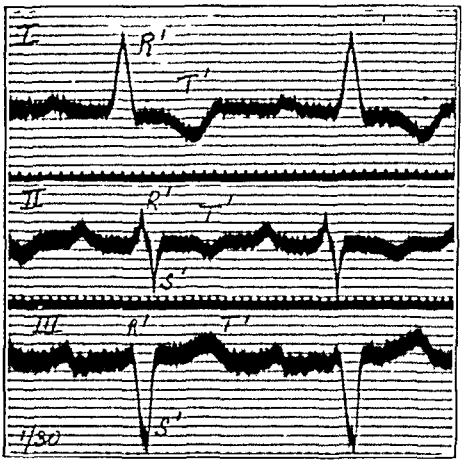


Fig. 6.—Electrocardiograms, Case 10. The *P R* interval measures 0.21 to 0.23 second. In Lead I the initial deflection *R'* appears as a slightly broadened peak with notching of the side and apex. *T'* is inverted lying in the opposite direction to the summit *R'*. In Lead II, the initial deflection *R'* is conspicuously notched. The deflection *S'* is plainly seen. *T'* it will be noted is again inverted, lying in a direction opposite to *R'*. In Lead III, the greatly increased amplitude of the initial deflection *S'* is apparent. *S'* shows marked splitting of its base. *R'* is inconspicuous. *T'* is upright lying in a direction opposite to the prominent initial deflection *S'*. The increase in the *P R* interval is apparent in all leads.

carotids pulsate vigorously and the veins of the neck are abnormally prominent. The liver is not enlarged. The urine contains no albumin. The pulse is of the water-hammer type and occasionally intermits. Capillary pulsation is present. The arteries are thickened. The systolic blood-pressure lies between 180 and 200 mm. Hg.

Heart.—The visible impulse is diffuse. The upper limit of cardiac dulness is at the second rib, the limit of dulness to the right is 1½ inches and to the left 7½ inches from the midsternal line. At the apex the first sound is replaced by a systolic bruit, followed by an early diastolic murmur. At the aortic cartilage a diastolic thrill and murmur are detected, the second sound is absent.

Course.—The patient was under observation for over twelve months, during which period the physical signs and symptoms remained unaltered. With rest the pain disappeared, only to reappear as soon as the patient was about again.

He died suddenly in August, 1912 No post-mortem examination could be obtained

Electrocardiograms—The electrocardiographic curves were constantly of the same type, being those associated with deficient conduction of the right branch of the auriculoventricular bundle In this case extraventricular systoles were recorded appearing in a direction opposite to the prominent initial deflections in all leads, hence the premature beat arose in the opposite ventricle from the aberrant beat, that is, from the right and basal portion of the heart

CASE 11—*Arteriosclerosis, myocarditis, aortic disease, cardiac failure and death* (Electrocardiograms Fig 7)

Patient—C H, a seaman, aged 47, was admitted to the hospital July 20, 1912, complaining of shortness of breath and swelling of the legs

Family History—A brother is said to have had rheumatic fever, otherwise the family history is negative

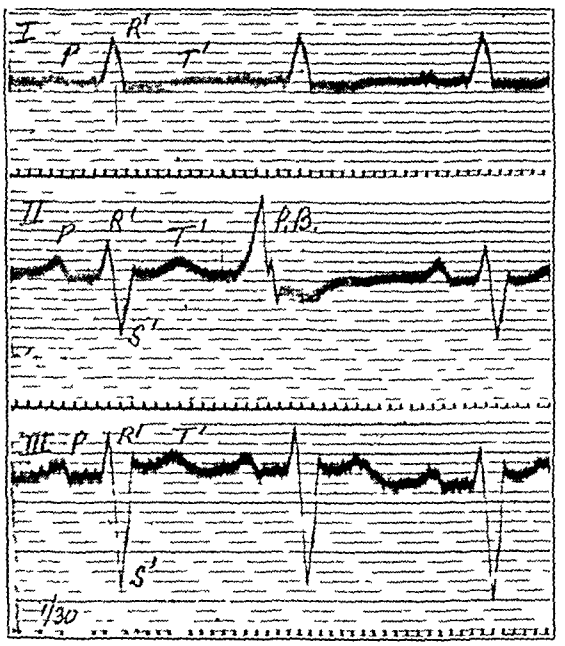


Fig 7—Electrocardiograms, Case 11 The *P R* interval measures 0 2 second In Lead I the initial deflection *R'* appears as a somewhat broadened summit with marked notching *T'* is upright in the same direction In Lead II, the initial deflection *R'* is plain The deflection *S'* shows an increase in amplitude and conspicuous notching *T'* is upright lying in a direction opposite to the prominent *S'* The single premature beat recorded in the figure—*P-B*—coincides with the auricular summit *P*, which does not appear The premature beat lies in a direction opposite to the prominent aberrant deflection *S'* In Lead III, the initial deflection *R'* is present The deflection *S'* appears as a conspicuous depression of greatly increased amplitude, with slight notching *T'* is upright in a direction opposite to the prominent deflection *S'* The premature beats recorded in this lead (none is shown in the figure), were in the same direction as the aberrant curve

Personal History—There is no history of any illness, with the single exception of an attack of "bronchitis," which the patient had eighteen months ago He has never had rheumatic fever He denies syphilis

Present Illness—The patient attributes the onset of his present illness to the "bronchitis" which he had eighteen months ago This attack was soon followed by shortness of breath, which has increased steadily During the past weeks his legs and abdomen have become swollen

Condition on Admission—The patient is florid The conjunctivae are tinged with bile He is cyanotic and very orthopneic There are signs of fluid in the right chest There is marked edema of the back, legs and scrotum, and abdomi-

nal ascites is present The liver is palpable below the umbilicus and pulsating The respirations are 32 to the minute The temperature is normal The specific gravity of the urine 1 012, the normal amount is reduced and albumin is present in excess The pulse rate is from 118 to 120 per minute, occasionally intermittent, of the water hammer type Capillary pulsation is present The arteries are thickened The systolic blood pressure is low, from 98 to 102 mm Hg

Heart—The point of maximum impulse is neither visible nor palpable The upper limit of cardiac dulness lies at the fourth interspace, to the right at the right sternal margin and to the left 4½ inches from the midsternal line There is an apical systolic murmur and a faint first sound At the aortic area systolic and diastolic murmurs are heard

Course—The patient was given infusion of digitalis (2 drams three times daily), but showed no improvement He died July 27, a week after admission Necropsy was refused

Electrocardiograms—In three observations, extending over a period of six weeks, the *P-R* interval measured 0 26, 0 16 and 0 2 second, the last two days before death The curves were always of the same type, being those seen in association with defective conduction of the right branch of the auriculoventricular bundle The extra systoles recorded were in a direction opposite to the prominent initial deflection *S'*, of the aberrant curve, in Lead II, but in the same direction as the aberrant curve in Lead III (Fig 7)

CASE 12—*Myocarditis and aortic disease Febrile auriculoventricular heart-block of varying degree* (Electrocardiograms, Figs 8 and 9)

Patient—W C, a bookbinder, aged 32, was admitted to the hospital Aug 16, 1912, complaining of shortness of breath and pains in the chest

Personal History—*The patient states that he always felt perfectly well* until about six years ago He has never had rheumatic fever He denies syphilis He is a heavy smoker, but uses alcohol in moderation

Present Illness—The patient dates the onset of his present illness back about six years, when he first began to notice shortness of breath after any unusual exertion This shortness of breath has increased, and for some months now he has had pain of an aching character over the precordial area He is unable to lie on the right side at night

Condition on Admission—The patient, thin and pale, lies propped up in bed and quite breathless There is no dropsy or liver enlargement The urine contains no albumin The pulse is regular, of the water hammer type, ranging from 64 to 88 per minute The arteries are thickened The systolic blood pressure varies from 110 to 148 mm Hg The temperature is 99 F

Heart—The heart's impulse is diffuse in the fourth, fifth and sixth interspaces The limits of cardiac dulness lie at the third rib above, ¾ of an inch to the right and 5½ inches to the left of the midsternal line At the apex a short, sharp first sound and a low pitched presystolic murmur are heard The second sound is faint and is followed by an early diastolic murmur At the base a systolic thrill is felt, and rough to and-fro murmurs are audible, having their maximum intensity at the aortic cartilage The second sound is not heard

Course—The temperature rose to 100 5 F the day after admission, but fell gradually to normal by August 22 After several weeks rest, his symptoms being considerably improved, he was discharged

Readmission (May 27, 1913) The patient complains of throbbing pain in the chest, which has increased during the past three weeks

Condition on Readmission—There is no cyanosis or dyspnea The pulse is regular in force and rhythm, of water hammer type Capillary pulsation present The arteries are sclerosed, as noted formerly

Heart—The cardiac dulness lies at the third rib above, to the right at the right sternal margin, to the left 5½ inches from the midsternal line There

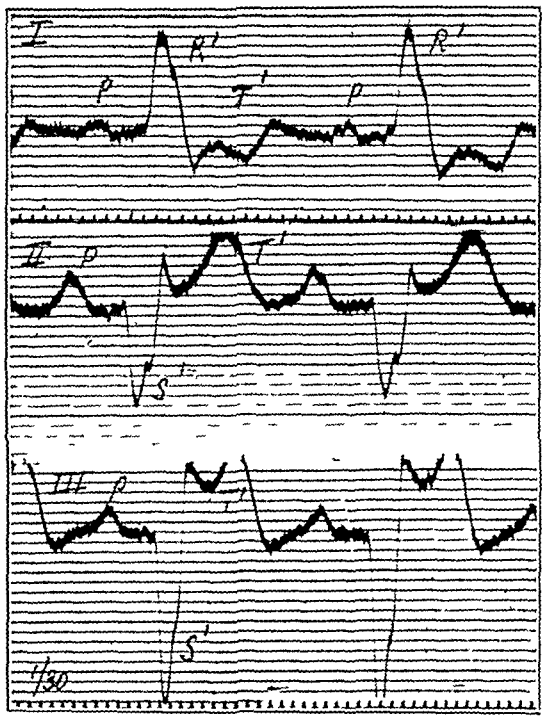


Fig 8—Electrocardiograms, Case 12 The *P-R* interval measures 0 18 second The *Q-R-S* interval measures 0 13 second In Lead I the initial deflection *R'* appears as a broad, tall peak with conspicuous notching *T'* is inverted lying in a direction opposite to the prominent deflection *R'* In Lead II *R'* is wanting, the initial deflection *S'* appears as a deep depression of increased duration as well as amplitude, with conspicuous notching *T'* is exaggerated, upright, in a direction opposite to the prominent deflection *S'* In Lead III *S'* is conspicuous as a deflection of greatly increased amplitude with marked notching *T'* is upright in a direction opposite to that of the conspicuous deflection *S'* The increase in the *P-R* interval and the great increase in the duration and amplitude of the initial phases of the ventricular complex is apparent in all three leads The bizarre character of the curves recorded on this date is in striking contrast to the curves taken on the following day

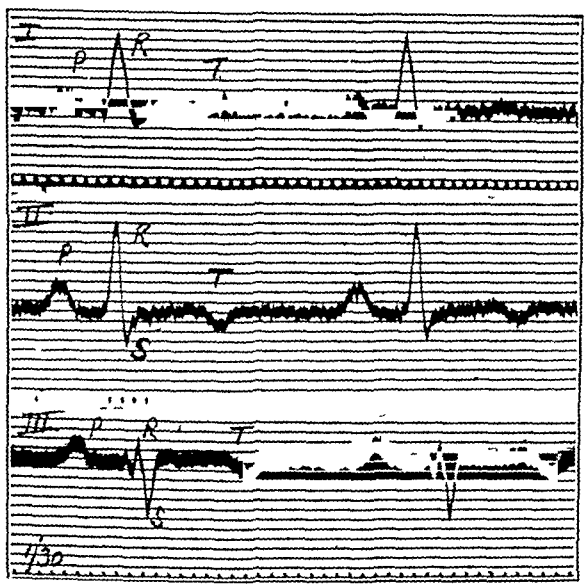


Fig 9—Electrocardiograms, Case 12 The *P R* interval measures 0 18 second The *Q R-S* interval measures less than 0 1 second In Lead I, *R* is present as a peak of short duration and amplitude *T* is upright, lying in the same direction as the peak *R* In Lead II, *R* and *S* are present R appearing as a deflection of short duration *T* is inverted lying in a direction opposite to the peak *R* In Lead III the initial deflections *R* and *S* are not exaggerated *T* is again inverted

is a systolic as well as a presystolic murmur at the apex, the latter is heard only with every fifth or sixth beat Over the aortic area there is a double murmur The specific gravity of the urine is 1 028, a trace of albumin is present

June 12, 1913 Under rest the patient's condition, so far as subjective symptoms are concerned, has materially improved His pain has been relieved and he feels much more comfortable The physical signs remain unchanged

Electrocardiograms—This case is of especial interest, in that during the period the patient has been under observation, varying degrees of heart block have been noted

A comparison of the electrocardiographic curves taken on the second and third day after his first admission (August, 1912) is most interesting and instructive, the exaggerated, bizarre form recorded August 18 (Fig 8), the result of defective conduction of the right branch of the auriculoventricular bundle, due to his febrile attack, giving place to the very different curve recorded on the next day (Aug 19, 1912) On this date the ventricular portions of the curves have entirely altered (Fig 9) There is no evidence of any defect in conductivity of the right branch of the bundle, but only of a relative preponderance of the left ventricle

These two figures are most instructive, illustrating as they do the occurrence of a temporary defect in conductivity of the right branch of the auriculoventricular bundle (Fig 8), and on the subsidence of this acute defect, the physiological electrocardiograms for this patient (Fig 9) indicative only of a relative left ventricular hypertrophy In addition to the very apparent differences in the duration and amplitude of the opening phases, as well as in the direction of the prominent deflections in the various leads of the two figures, a comparison of the direction of the summits T' and T in the corresponding leads is most important In each of the corresponding leads, of the two figures, it will be noted that where T' points in one direction T points in the other, and that, throughout, T is much less conspicuous than T'

In Figure 8, T', in accordance with the general rule in these cases, lies in a direction opposite to that of the prominent initial deflections in all leads In Figure 9 the inversion of T in Lead II is in itself of pathological significance

Note the absence of notching in the ventricular complex of Figure 9

These two figures may well serve to emphasize the differences between the curves obtained as a result of a defect in conductivity of the right branch of the auriculoventricular bundle, and those due to a relative hypertrophy of the left ventricle

CASE 13—*Rheumatic myocarditis, pleurisy with effusion, displacement of the heart and cardiac failure* (Electrocardiograms, Fig 10)

Patient—R A, aged 35, was admitted to the hospital Sept 11, 1912, complaining of shortness of breath and swelling of the legs

Family History—Negative

Personal History—At the age of 18 the patient was ill for three months with acute rheumatic fever Subsequently he had many similar, though less severe attacks, until he was 28 Five months ago he had an acute pleurisy with effusion, was tapped and a large amount of fluid was withdrawn from the left pleura He has used alcohol in moderation and does not use tobacco

Present Illness—During the past seven years the patient has been quite free from any symptoms of acute rheumatic fever, but states that for the past seven months he has suffered from a feeling of exhaustion after slight exertion, and from very marked shortness of breath since his pleurisy five months ago He further states that, to his knowledge, his heart has been affected for ten years at least

Condition on Admission—The patient is thin and poorly developed He has a bad pyorrhea He is slightly cyanotic, and dyspnea is marked On the left side the movements of the chest are limited and tubular breathing is heard at

the apex behind There is dulness at the base of the left lung and the breath-sounds are weak Friction sounds are heard in the axillary region There is slight edema of both legs The temperature is normal The pulse is regular, from 76 to 104 per minute, the arteries are a little thickened, the systolic blood-pressure varies from 98 to 134 mm Hg

Heart—The point of maximum impulse lies in the fourth interspace, 2 inches inside the right nipple line There is a diffuse pulsation over the right side of the chest internal to the main impulse The limits of cardiac dulness lie at the third rib above, and 2½ inches to the right and left of the midsternal line The first sound is inaudible at the apex, the second sound is accentuated and a coarse systolic murmur is heard The second sound is reduplicated at both pulmonary and aortic cartilages A pleuropericardial friction-rub is heard over the left half of the precordium Roentgenoscopy showed great displacement of the heart to the right, with enlargement, but no trace of fluid could be seen

Subsequent History—After a sufficient period of rest in the hospital the patient so far improved as to be able to leave So far as is known at present (June 14, 1913) he is still living

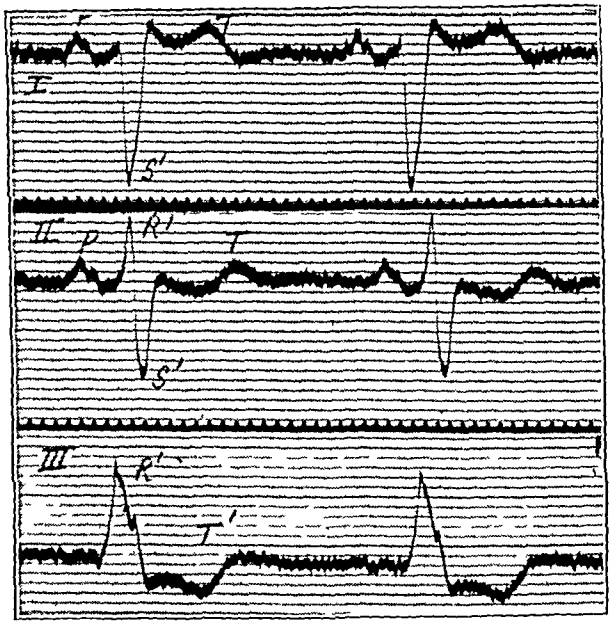


Fig 10—Electrocardiograms Case 13 The *P R* interval measures 0 13 second The *Q R S* interval measures 0 13 second In Lead I, *R'* is absent, the initial deflection *S'* appearing as a depression of greatly increased amplitude (apex negativity) with suggestive notching at the extreme base The summit *T'* is upright, lying in a direction opposite to the prominent deflection *S'* In Lead II, *R'* is present but the deflection *S'* is of greater amplitude and duration, again with suggestive notching of the base *T'* is upright in a direction opposite to the prominent deflection *S'* In Lead III, the initial deflection *R'* is present as a conspicuous summit of increased duration and amplitude, with marked notching *T'* is again conspicuous, inverted, lying in a direction opposite to the prominent initial deflection *R'* When compared with all the other accompanying figures, it will be seen that the direction of the prominent initial deflections in Leads I and III are in the opposite direction, It has been shown that in lesions involving the left branch of the auriculoventricular bundle the initial deflection in Lead I is always downward (apex negative) while in Lead III, it is always upward (base negative)

This case is of especial interest in that it is the single instance, in the series, of involvement of the left branch of the auriculoventricular bundle

CASE 14—*Arteriosclerosis, cardiac hypertrophy, myocarditis, alternation, cardiac failure and death*

Patient—W P, a waterside laborer, aged 62, was admitted to the hospital Nov 1, 1912, complaining of faintness and shortness of breath

Personal History—With the exception of an attack of pleurisy twelve years ago there is no history of any acute illness The patient has had repeated attacks of tonsillitis to which he has paid little attention He also states that he has suffered from so called "muscular rheumatism" for many years, but that he had not had acute rheumatic fever A sister is said to have had a history of vague "rheumatic" pains He denies syphilis He has used but little alcohol and smokes in moderation

Present Illness—For the past eighteen years the patient has felt less fit for work than previously, and from time to time has experienced shortness of breath Eight years ago while carrying heavy wheat sacks he had an attack of faintness and was forced to stop work He noticed at this time that his scrotum was a little swollen and that it increased in size during the next two days He was treated at a hospital, improved and went back to work A year later he had a similar attack and was again incapacitated He has not been well since this attack in 1906, and has been treated at several institutions recently for an aggravation of the same symptoms and faintness

Condition on Admission—The patient is pale and spare The conjunctivae are bile stained, there is a little cyanosis of the mucous membranes He has hurried and irregular breathing Dropsy of the legs, genitalia and scrotal region is present The urine is acid, specific gravity 1 018, and a trace of albumin is present The liver is enlarged and there is some ascites There is dulness at the left base of the chest with weak breath sounds, faint râles are heard at both bases The temperature is normal

Heart—The heart's dulness extends 2 inches to the right and 6½ inches to the left of the midsternal line There are no murmurs The heart-beats are either regular at 80 or 90 per minute, when cantering rhythm is heard, or the beats are in couples, when three sounds accompany each beat of the couple When the coupled beats are present the pulse rate is halved The arteries are thickened, the systolic blood-pressure is 120 mm Hg Venous pulsation in the veins is conspicuous and these vessels are enlarged

Course—After a prolonged stay in the hospital, the dropsy and many of the signs of engorgement were relieved, and the breathing was much freer The patient died Feb 13, 1913, shortly after leaving the hospital

Electrocardiograms—The P-R interval could not be determined owing to the presence of auricular fibrillation The ventricular complexes were similar to those shown in Figure 3 The premature beats recorded were in all leads—I, II and III—in a direction opposite to the aberrant beat, and hence arose from the right and basal portion of the ventricle

CASE 15—*Myocarditis in gouty subject, anginal symptoms, syncopal attacks, extrasystoles and alteration* (Electrocardiograms, Fig 11)

Patient—J W, a man, aged 68, was first seen Nov 6, 1912, complaining of attacks of faintness and occasional loss of consciousness

Personal History—For many years the patient has been subject to acute attacks of gout For a few years he has had occasional attacks of faintness and throbbing in the chest and a feeling of great distress, these symptoms coming on always after exertion In some of the attacks he has lost consciousness for a few seconds These attacks have not been frequent, and as a rule have been isolated, but on one occasion five attacks occurred one after another The feeling of distress is relieved by nitroglycerin

Condition on Admission—The patient is heavily built and shows little or no dyspnea or cyanosis The urine contains no albumin and there are no signs of dropsy The pulse intermits occasionally, the rate is 78 to the minute The systolic blood presure is 160 mm Hg

Heart—The impulse is diffuse, there is an extension of dulness towards the left side No murmurs are heard, but the first sound is reduplicated at the apex and base

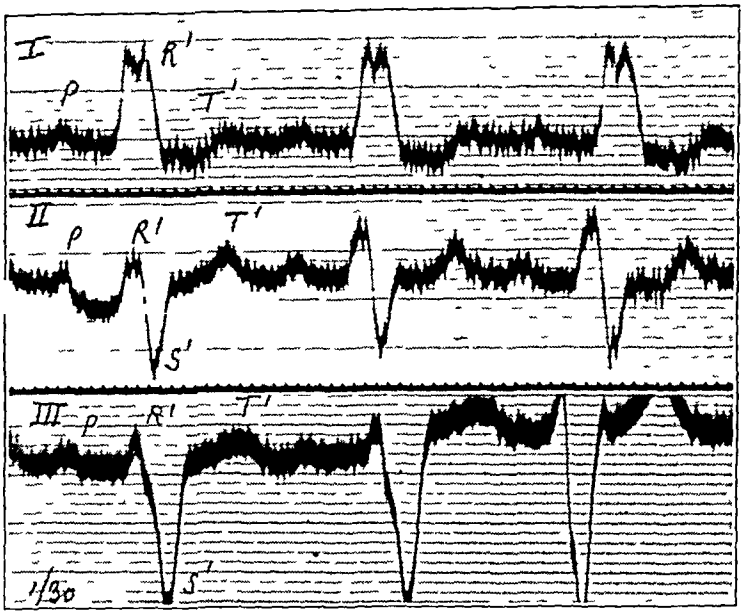


Fig 11 —Electrocardiograms Case 15 The $P\,R$ interval measures 0 2 second The Q-$R\,S$ interval measures 0 16 second In Lead I, the initial deflection R' appears as a broad wide summit, with conspicuously split top The final deflection T'' is inverted lying in a direction opposite to the deflection R' In Lead II, R' is present as a short summit with marked notching, the deflection S' is conspicuous as a deep depression with notching, the final deflection T'' is upright lying in a direction opposite to the prominent deflection S' In Lead III, R' is inconspicuous, S' appears as a deep depression of exaggerated amplitude and duration, with marked notching The final deflection T'' is again upright, lying in a direction opposite to the prominent deflection S' The increase of the $P\,R$ interval and the increase in the duration and amplitude of the early phases of the complex is apparent in all leads The single premature beat recorded in Lead III is in the same direction as the prominent deflection S'

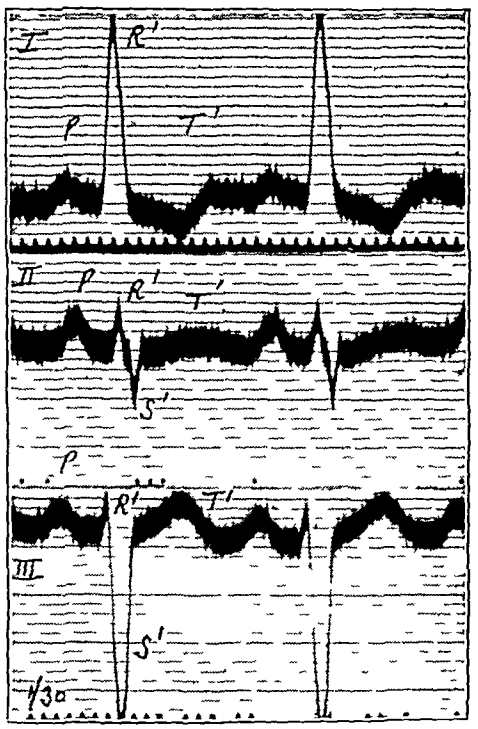


Fig 12 —Electrocardiograms, Case 16 The $P\,R$ interval measures 0 14 second The $Q\,R\,S$ interval is not increased, measuring 0 08 second In Lead I the initial deflection R' is conspicuous as a tall peak of greatly increased amplitude The final deflection T'' is inverted lying in a direction opposite to the prominent summit R There is no notching In Lead II, the initial deflection R' is present and equally as conspicuous as the deflection S' The final deflection T'' is upright In Lead III, the initial deflection R is inconspicuous, the deflection S' appearing as a deep depression of greatly increased amplitude The final deflection T' is upright in a direction opposite to the conspicuous deflection S' The increased amplitude of the initial deflections in Leads I and III is apparent, as is the direction of the final deflection T'' in relation to R' and S'

Graphic Records—The intermissions are the result of premature contractions arising in the ventricle They are either isolated or occur in short groups The premature beats are followed by alternation of the pulse

CASE 16—*Myocarditis, alternation, Cheyne Stokes breathing, high blood pressure and cardiac failure* (Electrocardiograms, Fig 12)

Patient—D E, a physician, aged 54, was seen Nov 16, 1912, complaining of breathlessness on exertion

Personal History—The patient had an attack of erysipelas when 15 Otherwise, with the single exception of an attack of pleurisy some years ago, his history is negative He has been moderate in his use of alcohol, but has always eaten very heartily His life has been very active

Present Illness—For three months past he has suffered from breathlessness, which has been increasing steadily At present it is brought on by even slight exertion There has been no pain, soreness of the chest or palpitation

Condition on Admission—The patient is heavily built, florid and a little cyanosed He is of a nervous temperament There is slight dropsy of the legs, but no enlargement of the liver He has a tendency to Cheyne Stokes breathing The urine contains no albumin

Heart—The apex beat cannot be found The area of the heart's dulness is increased to the right and left At the apex, gallop rhythm is heard There are no murmurs At the base the sounds are normal The pulse intermits occasionally, the arteries are much thickened, the systolic blood pressure is 210 mm Hg

Subsequent History—The patient went home and died in August, 1913,

CASE 17—*Rheumatic myocarditis, cardiac hypertrophy, aortic disease, ventricular extrasystoles and cardiac failure* (Electrocardiograms, Fig 13)

Patient—W O, a hotel porter, aged 27, was admitted to the hospital Feb 4, 1913, complaining of pain in the chest, ankles and knees

Family History—Negative

Personal History—The patient had chorea at 11, and rheumatic fever at 17 Following the rheumatic fever, ten years ago, his heart was affected, and he has had shortness of breath ever since

Present Illness—The patient refers the commencement of his present acute symptoms to the sudden onset, about ten days ago (January 26), of a sharp and severe pain in the chest, in the region of the heart, which is aggravated by breathing or by coughing Three days later his ankles became swollen, red and tender, and subsequently his knees also The patient has been short of breath for the past ten years, but this has been much worse during the past week

Condition on Admission—The patient's temperature is 101 F Both knees and ankles are swollen, but not red or tender, he is sweating profusely The pulse is typically water hammer in character, showing occasional extrasystoles Capillary pulsation is plainly visible There is a marked pulsation of the vessels in the neck The arteries are not thickend No rheumatic nodules are present

Heart—There is pronounced heaving of the entire left side of the chest, and a distinct shock palpable in the epigastrium The upper limit of cardiac dulness lies at the third rib, to the right 1½ inches and to the left 6½ inches from the midsternal line The apex is well outside the left nipple line in the fifth interspace The first and second sounds are reduplicated, the first over a wide area heard 3 inches to the right in the fifth interspace Over the apex there is a systolic murmur Over the aortic area there is a systolic and a diastolic murmur, the latter being conducted over the entire heart On admission there were signs of pleurisy with effusion at both bases, and a general bronchitis The liver was palpable 1½ inches below the costal margin, no pulsation was present

Course—By February 14 the temperature had fallen to normal, the swelling of the joints had subsided, the signs of effusion in the chest had wholly disappeared, and the dyspnea was greatly relieved He was later discharged from the hospital

Subsequent History (June 18, 1913)—The patient is alive and in fair condition

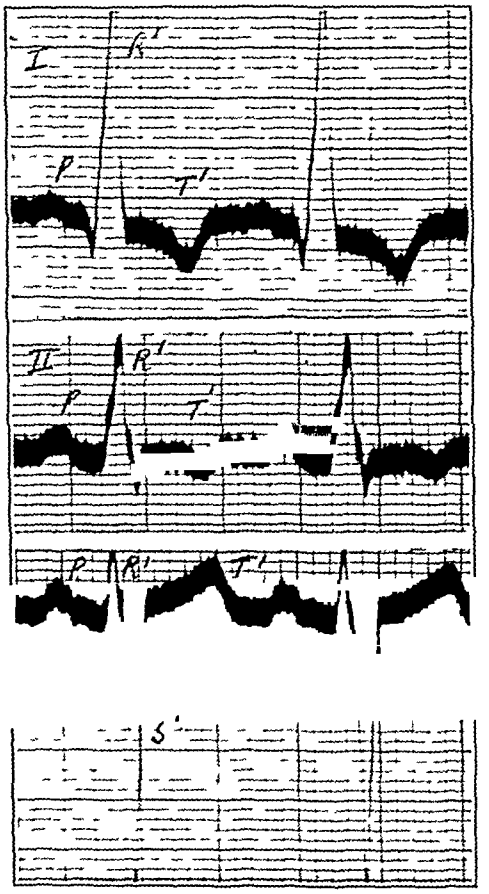


Fig 13—Electrocardiograms Case 17 The *P R* interval measures 0 17 second The *Q-R S* interval measures 0 08 second In Lead I, the initial deflection *R'* is conspicuous is a tall peak of greatly increased amplitude The final deflection *T'* is inverted, lying in the opposite direction In Lead II, the initial deflection *R'* is conspicuous as a summit of exaggerated amplitude with marked notching *S'* is inconspicuous The final deflection *T''* is again inverted, lying in a direction opposite to the prominent deflection *R'* In Lead III, the deflection *R'* is present as a short notched peak The deflection *S'* is conspicuous as a depression of enormously exaggerated amplitude The final deflection *T* is upright in a direction opposite to the conspicuous deflection *S'* The greatly increased amplitude of the initial deflections is apparent at a glance, as is the direction of the conspicuous final deflection *T''* in relation to the prominent deflections *R'* and *S'*

This case is of especial interest in view of the varying degrees of heart-block noted, the *P-R* interval varying between 0 28 and 0 17 second in curves taken on different occasions

CASE 18—*Arteriosclerosis, cardiac hypertrophy, aortic disease, acute pleurisy, cardiac failure and death* (Electrocardiograms, Fig 14)

Patient—G C, a milk-carrier, aged 47 was admitted to the hospital Feb 7, 1913, complaining of shortness of breath, sleeplessness and pain in the chest

Family History—Negative No history of rheumatic fever in the family

Personal History—There is a history of syphilis twenty-four years ago, at the age of 23, and of a severe attack of typhoid fever twelve years ago There is no history of rheumatic fever or of any other illness The patient has used alcohol and tobacco in moderation

Present Illness—About 18 months ago (August, 1911), the patient first began to experience attacks of pain, stabbing in character, starting in the front of the chest over the lower end of the sternum and passing through to the back between the shoulder blades These attacks came on chiefly at night, and lasted from half an hour to two hours The pain was often worse just under the left shoulder blade, and the cessation of the pain was frequently followed by paroxysms of breathlessness A week after the onset of these symptoms he noticed that his feet began to swell, and a few days later he was obliged to give up work During the past six weeks he has had frequent attacks of distressing shortness of breath associated with this pain in the chest, coming on even though lying quietly in bed, he has also had a troublesome cough unaccompanied with any expectoration

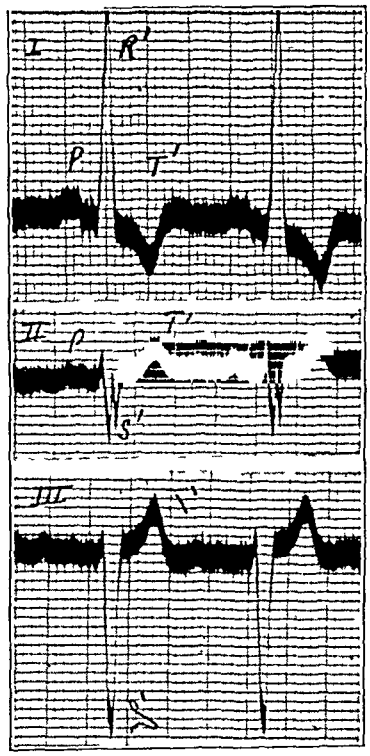


Fig 14—Electrocardiograms, Case 18 The *P-R* interval measures 0 2 second In Lead I, the initial deflection *R'* is conspicuous as a tall peak of greatly increased amplitude The final deflection *T''* is conspicuous and inverted, lying in a direction opposite to the prominent summit *R'* In Lead II, the deflection *R'* is absent, the initial deflection *S'* is conspicuous as a markedly split depression The final deflection *T''* is upright lying in a direction opposite to the prominent deflection *S'* In Lead III, the initial deflection *S'* is conspicuous as a deep depression of greatly increased amplitude The final deflection *T''* is upright, lying in a direction opposite to the prominent deflection *S'* The diphasic character of the complex in Leads I and III, the greatly increased amplitude of the initial deflections, and the direction of the conspicuous final deflection *T''*, in relation to the prominent initial deflections, are apparent

Condition on Admission—The patient, a well developed man, is lying propped up in bed, quite dyspneic The mucous membranes are pale There is marked pulsation of the vessels in the neck The pulse is full and regular in force and rhythm, not compressible, typically water hammer in character when the arm is raised The vessel wall is thickened

Heart—The cardiac impulse is visible in the fifth, sixth and seventh interspaces In the fifth interspace in the anterior axillary line it is heaving There is a systolic retraction 1 inch below the left nipple The upper limit of cardiac dulness lies at the third interspace, to the right 1¾ inches and to the left 6½

inches from the midsternal line At the apex a soft double murmur can be heard over a wide area Both of these murmurs can be followed up the left border of the sternum At the aortic cartilage the first sound is obscured by a harsh systolic murmur transmitted upward into the vessels of the neck The second sound is distant and followed by an early diastolic murmur transmitted downward The first sound is not heard anywhere

Respiratory System—Normal but for a few fine crepitations at both bases posteriorly

Liver—The liver is palpable a hand's breath below the costal margin The spleen is not palpable There is marked edema of both legs The specific gravity of the urine is 1 006, a trace of albumin is present but no sugar No casts seen

During subsequent observation up to the time of death, April 10, the patient's general condition varied but little from day to day By February 25 the edema of the legs had disappeared, only to return again later The pain in the chest was persistent, coming on in paroxysms which left him very dyspneic and greatly depressed

The cardiac signs remained practically unchanged, though by March 19 the diastolic murmur at the base had wholly obscured the second sound

About April 1, the pain in the chest became more pronounced, the attacks came on more often and persisted longer April 5 the patient complained of great pain in the lower right side, and a friction-rub was discovered, heard over the four lower interspaces on the right side behind April 6 there was well marked dulness over this area, associated with weak respiratory sounds and decreased vocal resonance and fremitus On this day a small amount of bright red blood was coughed up This condition persisted and April 8 a friction-rub was audible over the fifth interspace on the left side anteriorly On the morning of April 10 a well marked pericardial friction rub was heard There was dulness below the angle of the right scapula, over which area the breath sounds were weak and the resonance increased The patient died suddenly during the morning The temperature just before death was 98 F, and at no time since admission had it been above 99 F

The systolic blood-pressure was 190 mm Hg on admission, and had ranged between this figure and 140 mm Hg, the mean systolic pressure being about 140 mm Hg The specific gravity of the urine ranged from 1 006 to 1 028 A trace of albumin was constantly present No casts were seen

CASE 19—*Arteriosclerosis, high blood pressure, cardiac hypertrophy, myocarditis, mitral disease, nephritis and cardiac failure* (Electrocardiograms, Fig 15)

Patient—W J, a coach builder, aged 42, was admitted to the hospital Feb 10, 1913, complaining of shortness of breath

Family History—Father died of pneumonia, aged 52 Mother died at 47 of a "rheumatic heart"

Personal History—There is no history of chorea or rheumatic fever He contracted syphilis in 1889, for which he was treated with mercury over a period of eighteen months During the winter of 1911 to '12 he had an attack of asthma He has used alcohol in moderation, tobacco to the extent of 2 ounces a week

Present Illness—The patient attributes the onset of his present acute symptoms to the attack of asthma, which he had a year ago Soon after this illness he began to suffer from shortness of breath and attacks of palpitation, which of late have grown more frequent and have been very distressing Eight months ago he began to cough and from this time has had a persistent cough accompanied by a frothy expectoration, at times slightly streaked with blood For the past two months he has noticed that he has voided less urine than usual He has slept poorly

Condition on Admission.—The patient is a stout man who looks older than he is. As he lies propped up in bed there is slight dyspnea. The respirations are 28. The chest moves but little and there is some impairment of resonance over the left base posteriorly. Over this area the respiratory sounds are weak. Sonorous and sibilant rales are heard over the entire chest. There is no clubbing of the fingers. The pulse is regular in force and rhythm, 100 per minute. The systolic blood pressure is 186 mm. Hg. The liver is palpable 3 inches below the costal margin. There is slight edema of the feet. The temperature is normal. The specific gravity of the urine, on admission, was 1.030, with albumin present in excess and many hyaline and granular casts. The Wassermann reaction is positive.

Heart.—The apex beat is visible and palpable in the sixth interspace, 6 inches from the midsternal line. The upper limit of cardiac dulness lies at the third interspace, to the right 1½ inches, and to the left 6 inches from the midsternal line. At the apex the first sound is loud and booming, accompanied by a soft systolic murmur, the second sound is short and valvular in character. The aortic second sound is accentuated, no murmurs are heard over the aortic area.

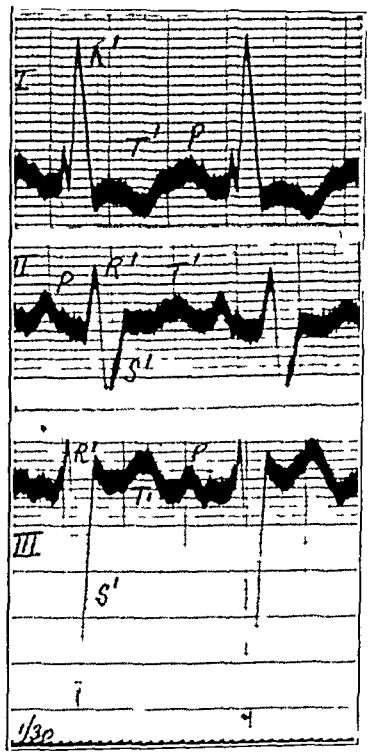


Fig. 15.—Electrocardiograms, Case 19. The *P R* interval measures 0.2 second. In Lead I, the initial deflection *R'* is conspicuous as a tall peak of increased amplitude, with conspicuous notching. The final deflection *T''* is inverted, lying in the direction opposite to the deflection *R'*. In Lead II, *R'* and *S'* are both present and both show notching. The deflection *S'* is slightly the more conspicuous. The final deflection *T''* is upright, lying in a direction opposite to *S'*. In Lead III, *R'* is present, but the deflection *S'* is conspicuous as a deep depression of greatly increased amplitude. The final deflection *T''* is upright, in a direction opposite to the prominent *S'*. The increased amplitude of the initial deflections, the increased duration of the opening phases—the *Q R S* interval measures 0.12 second—and the direction of the final deflection *T''*, in relation to *R'* and *S'*, are apparent.

Subsequent History.—After a few weeks rest the acute symptoms were much relieved and the patient left the hospital. He was readmitted at a later date and died in the hospital. The *P R* interval measures 0.18 and 0.2 second (two observations).

CASE 20.—*Myocarditis, cardiac hypertrophy, aortic disease, cardiac failure and death* (Electrocardiograms, Figs 16 and 17)

Patient.—D H, a laborer, aged 38, was admitted to the hospital Feb 25, 1913, complaining of shortness of breath and pain in the chest in the region of the heart

Family History.—Mother is said to have died of "heart disease" No history of rheumatic fever in the family

Personal History.—There is no history of any acute illness The patient has never had chorea or rheumatic fever He had syphilis fourteen years ago He has used alcohol in moderation

Present Illness.—The patient states that his present illness began five months ago, when he first noticed that he became short of breath after any slight exertion This was soon followed by a sense of fulness and oppression in the chest During the past month he has had attacks of pain in the chest referred chiefly to the region of the second and third interspaces on the left side These attacks have latterly become more frequent, and at present the pain is quite constant There has been no cough

Condition on Admission.—The patient is well-built, slightly cyanotic and quite dyspneic The pulse is regular in force and rhythm, typically water-hammer in character Capillary pulsation is present The liver is palpable below the costal margin

Heart.—The point of maximum impulse is diffuse in the sixth interspace almost at the anterior axillary line There is a palpable systolic thrill at the apex The upper limit of cardiac dulness lies at the third rib, to the right 1¾ inches and to the left 6 inches from the midsternal line Over the aortic area there is a double murmur, and passing upward a short systolic murmur is heard over the vessels in the neck Passing downward the diastolic murmur becomes louder, being most intense over the third and fourth interspaces As one passes outward toward the apex a rough rumbling murmur is heard just before and associated with doubling of the first sound, suggesting a Flint murmur

Course.—After several weeks the patient's general condition so far improved that he was able to leave the hospital, being at the time of his discharge practically free from pain, though still dyspneic upon exertion The area of cardiac dulness had slightly diminished, but otherwise the physical signs remained unchanged, though at this time the rumbling murmur heard at the apex was not clearly associated with reduplication of the first sound

Readmission (May 15, 1913).—The patient was readmitted to day, all his old symptoms being more pronounced While he was in the out patient department, the following note was made Breathlessness more marked, slight cyanosis and excessive urobilin staining There is a slight effusion at the base of the left lung The liver is palpable at the level of the umbilicus

Heart.—The apex-beat is diffuse in the sixth interspace The limits of cardiac dulness are to day at the third rib above, 2 inches to the right and 6½ inches to the left of the midsternal line There is a systolic thrill at the apex and a systolic and a full diastolic rumble are heard over this area Over the aortic and pulmonary areas there is a double murmur

Course.—The patient's general condition grew gradually worse and he died in June, 1913

Electrocardiograms.—This case is of more than usual interest in view of the widely varying degrees of heart block recorded The *P-R* interval ranged from 0 21 to 0 3 second A comparison of the electrocardiographic curves recorded February 27 (Fig 16) and again May 5, 1913, (Fig 17), is most interesting and instructive

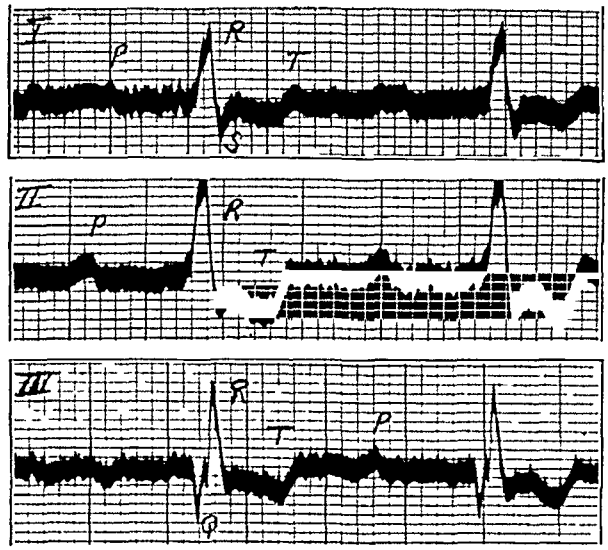


Fig 16.—Electrocardiograms Case 20 Before the development of the lesion involving the right branch of the auriculoventricular bundle The $P\,R$ interval measures 0 3 second In Lead I, Q is absent, R is prominent and already shows notching S is inconspicuous The final deflection T is upright In Lead II, the initial deflection R is more prominent than in the first lead The final deflection T is inverted In Lead III Q is conspicuous, R is prominent, and the final deflection T is again inverted The increased P-R interval, the suggestive notching of the prominent deflection R, and the inversion of T are the sole signs of a disturbed mechanism, the curve is essentially that of cardiac hypertrophy



Fig 17.—Electrocardiograms, Case 20 The $P\,R$ interval measures 0 22 second The $Q\,R\,S$ interval measures 0 12 second In Lead I, the initial deflection R' appears as a tall wide peak, of greatly increased amplitude, with marked notching The final deflection T' is inverted The premature beat lies in the same direction as R' In Lead II, the initial deflection R' appears as a conspicuously split summit The final deflection T' is upright The premature beat lies in a direction opposite to the conspicuous initial deflection R' In Lead III R' is absent the conspicuous initial deflection S' appears as a deep depression, of increased amplitude, and with marked notching The premature beats, it is seen, are in the same direction as the prominent initial deflection R' in Lead I, and in the opposite direction in Lead II

These two figures illustrate again, in a striking way, the difference between the electrocardiogram obtained as a result of simple cardiac hypertrophy, and that due to defective conductivity

The earlier physiological electrocardiogram for this patient, obtained in the light of a full knowledge of the degree of cardiac hypertrophy present, could not be confused with the aberrant complex obtained later, after the development of the lesion involving the conduction of the right branch of the bundle

CASE 21.—*Myocarditis, arteriosclerosis, aortic disease and cardiac failure* (Electrocardiograms, Fig 18)

Patient.—J M, a porter, aged 39, was first seen June 5, 1913, complaining of palpitation of the heart and shortness of breath

Family History.—Negative

Personal History.—There is no history of any acute illness The patient has not had rheumatic fever There is a history of syphilis fourteen years ago, for which he was under treatment nine months He uses alcohol and tobacco in moderation

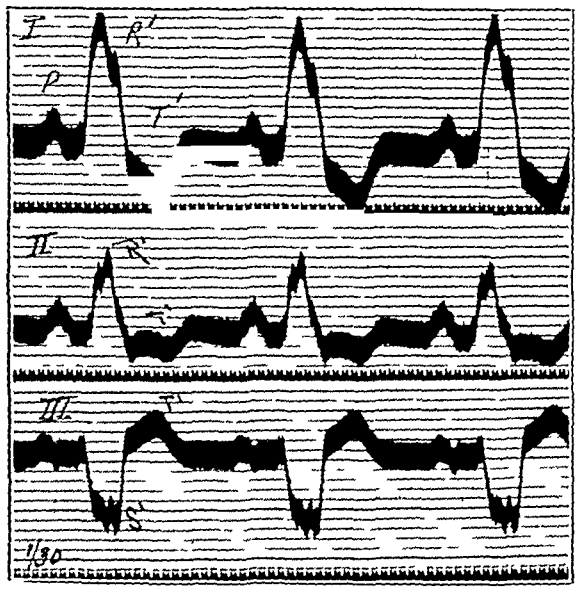


FIG 18.—Electrocardiograms, Case 21 The *P-R* interval measures 0 17 second The *Q R S* interval measures 0 2 second In Lead I, the initial deflection *R'* is conspicuous as a tall, broad summit with marked notching The final deflection *T''* is conspicuous and inverted, lying in a direction opposite to the summit *R'* In Lead II, the initial deflection *R'* is again conspicuous as a summit of increased height and duration, with marked notching The final deflection *T''* is inverted, lying in a direction opposite to the prominent summit *R'* In Lead III, the initial deflection *S'* is conspicuous as a deep, broad depression with marked notching The final deflection *T''* is upright in a direction opposite to the conspicuous *S'* The increased amplitude of the initial deflections, the increased duration of the opening phases, the direction of the final deflection *T''* in relation to the prominent initial deflections *R'* and *S'*, the conspicuous notching, and the diphasic character of the complex are apparent

Present Illness.—The patient states that for the past seven years he has had palpitation of the heart on slight exertion Beginning in August, 1912, he first noticed that he quickly became very short of breath after any unusual effort He has had occasional pains in the right shoulder, but nothing that could suggest acute rheumatic fever Under medical advice he has been doing little work for the past three months, and states that his general condition has improved considerably

Condition on Admission.—The patient is thin and rather poorly nourished He is slightly cyanotic and quite breathless on exertion The pulse is regular in force and rhythm, except for an occasional extrasystole The arteries are moderately thickened The systolic blood-pressure is 120 mm Hg

Heart—The point of maximum impulse is in the fifth and sixth interspaces The limits of cardiac dulness lie at the third interspace above, and 2½ and 5 inches to the right and left of the midsternal line, respectively There is a well-marked shock over the left side of the cardiac area and considerable pulsation in the second right interspace There is a systolic thrill at the base and a pronounced diastolic murmur at the aortic cartilage There is a suggestive Flint murmur at the apex The liver is just palpable at the costal margin The Wassermann reaction is positive

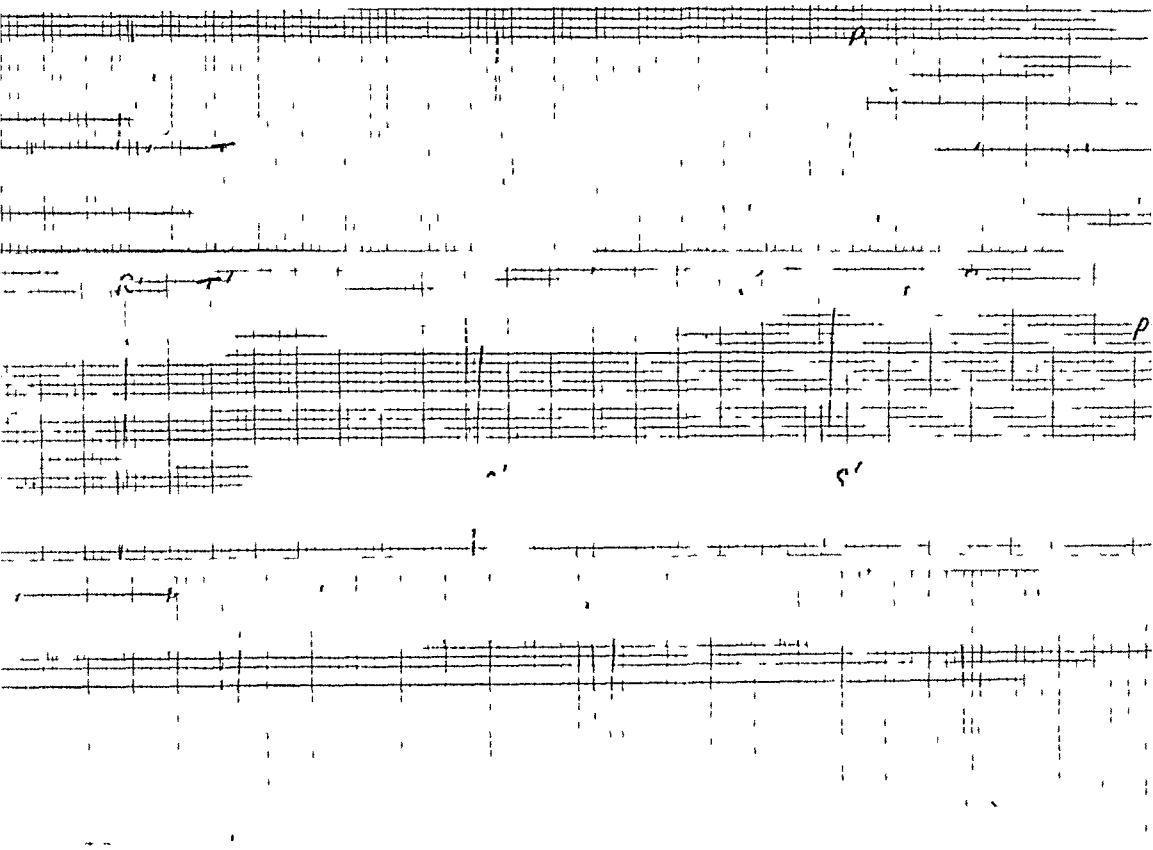


Fig 19—Electrocardiograms, Case 22 Note the complete independence of the auricular and ventricular rhythms The ventricular rate is 39, the auricular rate approximately 60 In Lead I, the initial deflection R' is conspicuous as a tall summit, with marked notching of increased amplitude and of slightly increased duration The final deflection T'' is conspicuous and inverted, lying in a direction opposite to the prominent summit R' The auricular summit P falls just at the conclusion of the deflection R' in the first complex of the figure, and obscures the diphasic character of the complex as seen in the second and third ventricular deflections In Lead II, the initial deflection R' is present but not prominent The deflection S' is conspicuous as a deep depression of greatly increased amplitude The final deflection T'' is inverted, lying in the same direction as the prominent deflection S In Lead III, R' is just seen The initial deflection S' is again conspicuous as a deep depression of greatly increased amplitude, with notching near the base The final deflection T'' is upright in a direction opposite to S' The $Q\,R\,S$ interval exceeds 0 1 second in all three leads The premature beats recorded in this case were in a direction opposite to the aberrant deflections in Leads I and III, and hence arose from the right and basal portion of the ventricle

CASE 22—*Congenital pulmonary stenosis, cardiac hypertrophy and complete auriculoventricular heart block* (Electrocardiograms, Fig 19)

Patient—E N, aged 27, a domestic, was first seen March 12, 1913, complaining of shortness of breath, of a "winter cough" for as far back as she can remember, and latterly of pain in the chest about the heart

Family History—Negative

Personal History—Apart from the statement, given above, of shortness of breath, and of the history of a "winter cough" for many years, there is absolutely no other history of any acute illness The patient has never had scarlet fever chorea, rheumatic fever or tonsillitis Careful questioning fails to elicit any history at all suspicious of a syphilitic infection She states that, as a young girl at school, she frequently used to "feel faint" but that she never fell down or lost consciousness She has had no such sensations of faintness now for many years She has had no palpitation or sense of disturbance as of sudden stoppage of the heart There is no history of any giddiness or dizziness in recent years

Present Illness—The patient has been in domestic service for the past seven years, doing hard, general work, which often involved heavy lifting She first began to notice more aggravated shortness of breath about a month ago (February 12), and found that she could not do her work as usual without quickly becoming very breathless and fagged out Three weeks ago (February 19) she first complained of pain in the chest, in the region of the heart of sudden onset, quite sharp, and described as jumping in character This pain is not constant, but comes on quite frequently three to eight, or more times a day The pain has never been referred to the left shoulder or down the left arm

Condition on Admission—The patient is slightly built and rather poorly nourished There is considerable cyanosis of the lips and hands, but no clubbing of the fingers The pulse is of small volume, regular in force and rhythm, 39 to the minute

Heart—The point of maximum impulse lies in the fifth interspace in the nipple line, but there is also a pulsation in the anterior axillary line The upper limit of cardiac dulness lies at the third interspace, to the right 1¾ inches, and to the left 5¼ inches from the midsternal line There is a pronounced shock over the precordium There is a well-marked thrill palpable over the upper precordium, and heard at its maximum over the second left cartilage At the apex the first sound is loud, followed by a soft systolic murmur The second sound is reduplicated Over the aortic area there is a faint systolic murmur The respiratory rhythm is almost synchronous with the ventricular rate, but tracings show that this is not constant

Course—The patient has been seen quite regularly, at weekly intervals Her general condition has improved By May 19 she was quite free from any attacks of pain, and the extreme breathlessness, for which she first sought relief, had been greatly relieved May 26 she felt so much better that she asked to be allowed to return to work

Electrocardiograms—This case is of more than unusual interest because of the presence of complete auriculoventricular heart-block together with a lesion involving the conductivity of the right branch of the auriculoventricular bundle It is the single instance of complete auriculoventricular heart-block in our series, and illustrates in an incomparable way, the association of lesions involving the main stem and one of its divisions

CONCLUSIONS

1 In the presence of lesions involving the conductivity of the branches of the auriculoventricular bundle, a distinct and characteristic type of electrocardiogram is obtained To the ventricular contractions on which these depend the term *aberrant* may be applied

2 This type of electrocardiogram is frequently associated with aortic valve disease, it is distinct from that of hypertrophy predominant in one ventricle

3 Bundle-branch defects are usually accompanied by defects in the main trunk, suggesting that the junctional tissues as a whole have a special pathology

4 The evidence in our possession is too slight to admit of any definite conclusions as to the relation between lesions of the bundle branches and the occurrence of extrasystoles

5 The electrocardiographic pictures of aberrant beats are probably of considerable prognostic importance

I wish to express my indebtedness to Dr Thomas Lewis, of London, for his kindness in placing his notes and records at my disposal, for many valuable suggestions and the courteous aid received from him in the preparation of this report

1872 East Ninety Seventh Street

THE ARCHIVES

OF

INTERNAL MEDICINE

EDITORIAL BOARD

JOSEPH L MILLER, Chicago

RICHARD C CABOT, Boston

THEO C JANEWAY, New York City

GEORGE DOCK, St Louis

W T LONGCOPE, New York City

W S THAYER, Baltimore

VOLUME XIII

1914

CHICAGO

AMERICAN MEDICAL ASSOCIATION

PUBLISHERS

CONTENTS OF VOLUME XIII

JANUARY, 1914 NUMBER 1

PAGE

Studies on the Circulation in Man X The Blood Flow in the Hands in Diseases of the Heart G N Stewart, M D, Cleveland 1

A Study of the Blood-Pressure in Children Showing Orthostatic Albuminuria Murray H Bass, M D, and H Wessler, M D, New York 39

Experiments to Develop a More Widely Useful Method of Blood Transfusion Henry S Satterlee, M D, and Ransom S Hooker, M D, New York 51

The Condition of the Blood in Hemophilia, Thrombosis and Purpura W H Howell, M D, Baltimore 76

A Clinical Study of Amylase in the Urine, with Special Reference to the Phenolsulphonephthalein Test H Rawle Geyelin, M D, New York 96

The Clinical Study of Hypercholesterinemia Harry B Schmidt, M D, Ann Arbor, Mich 121

The Urea Content of the Spinal Fluid with Special Reference to its Diagnostic and Prognostic Significance A Series of Ninety-Seven Cases Willard E Soper, M D, and Selma Granat, New York 131

The Gonococcus Complement Fixation Test and Analysis of Results From its Use B A Thomas, M D, and Robert Ivy, M D, Philadelphia 143

A Comparison of the Excretory Power of the Skin with that of the Kidney Through a Study of Human Sweat H W Plaggemeyer, M D, and E K Marshall, Jr, Ph D, Baltimore 159

Status Lymphaticus in Adults Its Clinical Diagnosis and Importance Haven Emerson, M D, New York 169

FEBRUARY, 1914 NUMBER 2

PAGE

Studies on the Circulation in Man XI The Blood-Flow in the Hands and Feet in Certain Diseased Conditions of the Vessels or of their Venous Mechanism G N Stewart, M D, Cleveland 177

Chronic Gastric Ulcer and Its Relation to Gastric Carcinoma Review of 684 Specimens William Carpenter MacCarty, M D, and Albert Compton Broders, M D, Rochester, Minn 208

The Non-Protein Nitrogenous Constituents of the Blood in Chronic Vascular Nephritis (Arteriosclerosis) as Influenced by the Level of Protein Metabolism Otto Folin, Ph D, W Denis, Ph D, and Malcolm Seymour, M D, Boston 224

Urobilin Its Clinical Significance Ray Lyman Wilbur, M D, and Thomas Addis, M D, San Francisco 235

The Effect of Heat on Blood Pressure L H Newburgh, M D, and C H Lawrence, M D, Boston 287

Paroxysmal Auricular Fibrillation G Canby Robinson, M D, St Louis, Mo 298

The Mutual Action of Certain Digestive Ferments J H Long, Sc D, and G W Muhleman, M S, Chicago 314

MARCH, 1914 NUMBER 3

PAGE

A Pulmonary Attack Simulating Primary Lobar Pneumonia, Caused by Pulmonary Embolism and Infarction from a Latent Venous Thrombosis Lewis A Conner, M D, New York 349

The Comparative Value of Cardiac Remedies Theodore C Janeway M D, New York 361

Interpretation of the Auscultation Blood Pressure Sounds D R Hooker, M D, and J D Southworth, B A, Baltimore 384

A Pathological Study of Two Cases of Heart Block with Adams Stokes Syndrome Edward B Krumbhaar, M D, Philadelphia 390

The Coagulation of Normal Human Blood An Experimental Study Roger I Lee, M D, and Beth Vincent, M D, Boston 398

Ulcus Ventriculi M H Gross, M D, and I W Held, M D, New York 426

A Skin Reaction Indicative of Immunitiy Against Typhoid Fever Studies in Typhoid Immunization III Frederick P Gay, M D, and John N Force, M D, Berkeley, Cal 471

The Influence of Lithium and Atophan on the Uric Acid Excretion of a Gouty Patient Amy L Daniels, Columbia, Mo 480

Comparative Study of Phenolsulphonephthalein Elimination and the Incoagulable Nitrogen of the Blood in Cardiorenal Diseases J Howard Agnew, M D, Ann Arbor, Mich 485

A Comparison of Methods of Obtaining Alveolar Air Walter M Boothby, M D, and Francis W Peabody, M D, Boston 497

Book Review Organic and Functional Diseases By M Allen Starr, M D, Ph D, LL D, Sc D, New York 507

APRIL, 1914 NUMBER 4

PAGE

Systemic Blastomycosis A Report of Its Pathological, Bacteriological and Clinical Features A M Stober, M D, Chicago 509

A Case of Systemic Blastomycosis R A Krost, M D, A M Stober, M D, Chicago, and M J Mods, M D, Dubuque, Iowa 557

A Case of Systemic Blastomycosis M Lewison, M D, and H Jackson, M D, Chicago 575

A Case of Systemic Blastomycosis H J Myers, M D, and A M Stober, M D, Chicago 585

A Case of Systemic Blastomycosis with Recovery T H Boughton, M D, Lawrence, Kan, and A M Stober, M D, Chicago 599

A Case of Systemic Blastomycosis H Jackson, M D, Chicago 607

A Case of Systemic Blastomycosis with Necropsy R E Bechtel, M D, and E R LeCount, M D, Chicago 609

A Case of Systemic Blastomycosis J S Eisentaedt, M D, and T H Boughton, M D, Lawrence, Kan 617

A Case of Systemic Blastomycosis Philip Frank Shaffner, M D 621

A Case of Systemic Blastomycosis T Churchill, M D, and A M Stober, M D, Chicago 568

A Case of Blastomycosis T H Boughton, M D, Lawrence, Kan, and S N Clark, M D, Hospital, Ill 594

A Case of Systemic Blastomycosis F B Riley, M D, and E R LeCount, M D, Chicago 614

Multiple Primary Malignant Tumors With a Report of Two Cases in Dogs Frank K Bartlett, M D, Chicago 624

Fatty Infiltration (Lipomatosis) of the Auriculoventricular Bundle of His, with Sudden Unexpected Death Frank Nuzum, M D, Chicago 640

Blood Pressures Their Relation to Each Other and to Physical Efficiency Joseph H Barach, M D, and W L Marks, M D, Pittsburgh, Pa 648

The Mechanism of Cardiac Displacements in Pulmonary Tuberculosis Maurice Fishberg, M D, New York 656

MAY, 1914 NUMBER 5

PAGE

ANIMAL CALORIMETRY PAPER VIII THE ALLEGED INFLUENCE OF THE ADRENALS ON DIABETIC METABOLISM GRAHAM LUSK AND J A RICHE, NEW YORK 673

EXPERIMENTAL STUDIES ON THE BLOOD SERUM OF COWS IMMUNIZED AGAINST TUBERCULOSIS SENSITIZATION OF LIVING TUBERCLE BACILLI EDWARD R BALDWIN, M D, SARANAC LAKE, N Y 682

AN EXPERIMENTAL STUDY OF INTESTINAL OBSTRUCTION JOHN A HARTWELL, M D, J P HOUGET, M D, AND FENWICK BEEKMAN, M D, NEW YORK 701

THE CLASSIFICATION OF AMEBAS WITH OBSERVATIONS ON MORPHOLOGY AND LIFE CYCLE OF ENTAMOEBA COLI, CRAIGIA HOMINIS AND VAHLKAMPFIA LOBOSPINOSA CHAS F CRAIG, M D, FORT LEAVENWORTH, KAN 737

THE PHYSIOLOGIC DEGENERATION OF THE MESONEPHROS WITH A CONSIDERATION OF ANTAGONISTIC ACTION OF ORGANS AND OF FUNCTIONAL MUTATION WITH MORPHOLOGICAL PERSISTENCE HORST OERTEL, LONDON, ENGLAND 770

A METHOD DEVELOPED FOR OBTAINING A STANDARD WASSERMANN ANTIGEN CYRUS W FIELD, M D, NEW YORK 790

A STUDY OF THE ACTION OF ATROPIN ON THE EOSINOPHIL CELLS OF THE BLOOD W W HERRICK, M D, NEW YORK 794

CLINICAL OBSERVATIONS OF DEFECTIVE CONDUCTION IN THE BRANCHES OF THE AURICULOVENTRICULAR BUNDLE A REPORT OF TWENTY TWO CASES, IN WHICH ABERRANT BEATS WERE OBTAINED EDWARD PERKINS CARTER, M D, CLEVELAND, OHIO 803

JUNE, 1914 NUMBER 6

PAGE

THE VALUE OF HEXAMETHYLENAMIN AS AN INTERNAL ANTISEPTIC IN OTHER FLUIDS OF THE BODY THAN URINE FRANK HINMAN, M D, BALTIMORE 841

HEXAMETHYLENAMIN HUGH MCGUIGAN, M D, AND C L V HESS, M D, CHICAGO 853

SOME RELATIONS OF THE BRAIN AND OF THE OLFACTORY APPARATUS TO THE PROCESSES OF IMMUNITY HENRY SEWALL, M D, DENVER 856

METABOLISM IN DEMENTIA PRAECOX ELLISON L ROSS, PH D, HOSPITAL, ILL 889

THE TITRATION OF WASSERMANN REAGENTS LOYD THOMPSON, M D, LITTLE ROCK, ARK 904

ATTEMPTS TO PRODUCE SCARLATINA IN MONKEYS CHARLES KRUMWIEDE, JR, MATTHIAS NICOLL, JR, AND JOSEPHINE S PRATT, NEW YORK 909

GERMAN MEASLES (RUBELLA) AN EXPERIMENTAL STUDY ALFRED F HESS, M D, NEW YORK 913

THE DIFFERENTIAL DIAGNOSIS OF THE INTESTINAL ENTAMEBAS OF MAN CHAS F CRAIG, M D, FORT LEAVENWORTH, KAN 917

STUDY XXI THE IMMEDIATE EFFECT OF REPEATED DOSES OF THEOBROMIN, SODIUM SALICYLATE AND THEOCIN ON RENAL FUNCTIONS IN ACUTE EXPERIMENTAL NEPHRITIS R FITZ, M D, BOSTON 945

THREE CASES OF ADAMS-STOKES SYNDROME WITH HISTOLOGICAL FINDINGS ADELE OPPENHEIMER, A M, AND B S OPPENHEIMER, M D, NEW YORK 957

THE INTRAMENINGEAL TREATMENT OF TABES AND CEREBROSPINAL SYPHILIS THOMAS R BOGGS, M D, AND R R SNOWDEN, M D, BALTIMORE 970

TWENTY-THREE CASES OF PNEUMONIA TREATED WITH ANTIPNEUMOCOCCUS SERUM WILLIAM R WILLIAMS, M D, NEW YORK 978

BOOK REVIEWS MATERIA MEDICA, PHARMACOLOGY, THERAPEUTICS AND PRESCRIPTION WRITING FOR STUDENTS AND PRACTITIONERS BY WALTER A BASTEDO, PH G, M D 987

PROTEIN SPLIT PRODUCTS IN RELATION TO IMMUNITY AND DISEASE BY VICTOR C VAUGHAN, M D, VICTOR C VAUGHAN, JR AND J WALTER VAUGHAN 987

CORRESPONDENCE A CORRECTION 988

The Archives of Internal Medicine

Vol XIII JUNE, 1914 No 6

THE VALUE OF HEXAMETHYLENAMIN AS AN INTERNAL ANTISEPTIC IN OTHER FLUIDS OF THE BODY THAN URINE *

FRANK HINMAN, M D
Assistant Resident Surgeon, Johns Hopkins Hospital
BALTIMORE

In previous communications I have presented a method for the quantitative estimation of formaldehyd in the urine of patients receiving hexamethylenamin by mouth,[1] and the results of a study with this method of the value of the internal use of hexamethylenamin as a urinary antiseptic.[2] This study on the urine clearly indicates that the conversion of hexamethylenamin into formaldehyd is a simple chemical process which will readily occur in an acid medium, but will not occur in an alkaline medium. It also shows that the amount of this conversion is dependent on the degree of urinary acidity, the duration of exposure to the action of this acidity and the percentage concentration of the drug in it. These facts have a significant bearing on the justification of the wide-spread therapeutic use of hexamethylenamin for other than vesical conditions. It has been recommended as an antiseptic for practically all of the body fluids, the only basis for which, aside from clinical observations, is the presence of the drug in these fluids after internal administration.

Although Butlerow[3] had prepared hexamethylenamin more than thirty years before, Nicolaier,[4] in 1894, introduced it into medicine, and demonstrated formaldehyd in the urine as its decomposition product. Richardson,[5] in 1899, confirmed Nicolaier's findings. These observations immediately popularized the drug as a urinary antiseptic. In 1907, Bucura[6] found free hexamethylenamin in human milk. A year later

* Submitted for publication Jan 28, 1914

* Read Jan 5, 1914, before the Johns Hopkins Medical Society, Baltimore

1 Hinman, Frank: Tr Am Assn Gen-Urin Surg, May 7, 1913.

2 Hinman, Frank: An Experimental Study of the Antiseptic Value in the Urine of the Internal Use of Hexamethylenamin, Jour Am Med Assn, 1913, lxi, 1601.

3 Butlerow: Ann d Chem u Pharm, 1860, cxv, 330.

4 Nicolaier: Deutsch med Wchnschr, 1895, xxi, 541.

5 Richardson: Jour Exper Med, 1899, iv, 19.

6 Bucura: Arch f exper Path u Therap, 1907, iv, 398.

Crowe[7] showed that it is excreted in the bile, pancreatic juice and synovial and cerebrospinal fluids, and he recommended its prophylactic and curative value in diseases of the meninges of the brain and spinal cord Barton,[8] in 1910, found hexamethylenamin in secretions of the middle ear, and since then much has been written relative to its antiseptic value in otitis media In the same year Hanzlik[9] showed that it is excreted in saliva, and a year later Armstrong and Goodman[10] found it in the sputa of tuberculosis and of pneumonia About the same time Gradle[11] demonstrated its presence in the anterior chamber of the eye, and in 1912 Whitman[12] found it in both the aqueous and vitreous humors These findings have been repeatedly confirmed All authorities are, therefore, agreed that hexamethylenamin is present after internal administration, in practically every fluid of the body

Because of the ready conversion into formaldehyd in the urine, it has been assumed by some investigators that it acts in a similar manner in these other fluids, while other observers have presumably regarded hexamethylenamin itself as of antiseptic value In the literature there is no evidence of free formaldehyd having been demonstrated definitely in any of these body-fluids (urine excepted) Hanzlik[9] was unable to find it in the saliva, Schmid and Schroeter[13] failed to find it in human milk, Burnam[15] could not demonstrate it in the cerebrospinal fluid or bile, and more recently Hanzlik and Collins[15] have stated positively that free formaldehyd is never present in saliva, milk, bile, cerebrospinal, synovial, pericardial and pleural fluids of man or in the vitreous or aqueous humors of animals The apparent confusion on this point is due to the character of tests employed In those cases in which free formaldehyd has been reported as present in these fluids after the ingestion of hexamethylenamin, the tests that have been used employ strong acids, which, by their action on the hexamethylenamin, liberate formaldehyd, which then reacts

7 Crow, J S Johns Hopkins Hosp Bull, 1908, xix, 109, ibid, xx, 102

8 Barton, W M The Elimination of Hexamethylenamin by the Mucous Membrane of the Middle Ear, Jour Am Med Assn, 1910, liv, 871

9 Hanzlik, P J The Excretion of Hexamethylenamin in the Saliva, Jour Am Med Assn, 1910, liv, 1940

10 Armstrong, J J, and Goodman, E H The Excretion of Salicylic Acid and Hexamethylenamin in the Sputum, Jour Am Med Assn, 1911, lvi, 1553

11 Gradle, W S Ophth Rec, March, 1911

12 Whitman Arch Ophthalmol, 1912, xli, 604

13 Schmidt and Schroeter Centralbl f Physiol u Path d Stoffwechsel, 1910, v, 129

14 Burnam, C F An Experimental Investigation of the Value of Hexamethylenamin and Allied Compounds, THE ARCHIVES INT MED, 1912, 324

15 Hanzlik, P J, and Collins, R J Hexamethylenamin The Liberation of Formaldehyd and the Antiseptic Efficiency under Different Chemical and Biological Conditions, THE ARCHIVES INT MED, 1913, xii, 578

As an example of this may be cited the results of Ibrahim,[16] who in 1910 presented a bacteriological study of the cerebrospinal fluid with reference to the inhibitory action of hexamethylenamin on different organisms (*Staphylococcus aureus, Bacillus coli, B typhi abdominalis* and *B pyocyaneus*) with these conclusions

1 After the internal administration of hexamethylenamin this can be demonstrated in the cerebrospinal fluid of infants

2 Hexamethylenamin in solution in cerebrospinal fluid forms, on standing at 37 C, formaldehyd

3 Hexamethylenamin gives in the cerebrospinal fluid at body temperature bactericidal action

4 On the grounds of this activity it appears justified to administer hexamethylenamin as a therapeutic measure in all forms of serous and purulent meningitis

Ibrahim did not recognize the significance of acidity in the liberation of formaldehyd He used three tests Nicolaier's[17] *Bromwasserprobe,* Jorrison's test with the use of acetic acid,[18] and Hehner's test (sulphuric acid) The first is a test for hexamethylenamin and the other two, because of the acid used, are unreliable With regard to the antiseptic findings the bacterial counts are not very convincing, and I was unable to confirm them in three cases in which, after the addition of about 0 05 gm hexamethylenamin to 5 c c of spinal fluid, there was no inhibition of either *Staphylococcus aureus* or *Bacillus coli* after seventy-two hours' incubation, as shown by the count after transferring one oese to an agar-plate, although the delicate tests (phloroglucin and amidol) for formaldehyd were present

The importance of acidity to the decomposition of hexamethylenamin has but just recently been recognized Wiggs[19] suggested its influence in the urine in 1910 Jordan,[20] a year later, reported that formaldehyd was not found in alkaline urines, and recently studies by Smith,[21] Hanzlik and Collins[15] and myself[2] have independently demonstrated the importance of the degree of urinary acidity in the use of hexamethylenamin as a urinary antiseptic The excellent paper of Hanzlik and Collins appeared not long ago, and, since it is in accord largely with the present study, a few of their conclusions will be given, and are as follows

Alkalies prevent, while acids facilitate the liberation of formaldehyd from hexamethylenamin in all body fluids

16 Ibrahim Med Klin, 1910, p 48
17 Nicolaier Ztschr f klin Med, 1899, xxxviii, 350
18 Jorrison Chem Ztz, 1897 290
19 Wiggs South Med Jour, December, 1910
20 Jordan Biochem Jour, 1911, v, 274
21 Smith, G G Bost Med and Surg Jour, May 15, 1913

The liberation of formaldehyd from hexamethylenamin in pathological fluids obeys the same laws as in other solutions, that is, it can only occur in acid reaction

After administration, hexamethylenamin is present, but does not liberate free formaldehyd in blood, cerebrospinal, pleural, pericardial and synovial fluids, vitreous and aqueous humors, and urine when truly alkaline

The beneficial therapeutic effects of hexamethylenamin depend on the liberated formaldehyd Such effects are to be expected principally, if not always, in acid urine only

The present work embodies the testing for formaldehyd of both normal and pathologic fluids of patients being fed hexamethylenamin with a study of the action of these fluids *in vitro* toward the liberation of formaldehyd when subjected to infection The main question at issue was to determine whether infection could alter the reaction of these normally alkaline fluids sufficiently to produce the liberation of formaldehyd, and, if so, whether there would be enough liberated to produce antisepsis

ANTISEPTIC VALUES OF FORMALDEHYD AND HEXAMETHYLENAMIN

A review of the work of other investigators and my own experiments[1] indicates that formaldehyd is a slow and a relatively weak germicide, but that, up to certain dilutions, it exerts a powerful inhibitory influence on bacterial development This power of inhibition varies slightly with different organisms A dilution of liquor formaldehydi of about 1 16,000 will cause complete inhibition of the growth of *Bacillus typhosus* for twenty-four hours, a dilution of about 1 6,000 will completely kill the organism in that time, about 1 30,000 dilution will partially restrain its growth, while a 1 40,000 dilution has no apparent effect unless the results are shown by means of plating and a systematic bacterial count when a slight diminution in the count is obtained Higher dilutions have no inhibitory effect

Hexamethylenamin has been shown repeatedly (Burnam, Jordan, Hanzlik and Collins) to have no germicidal or antiseptic power Hanzlik and Collins found that hexamethylenamin, to which 0 5 per cent ammonium hydroxid had been added to prevent the liberation of formaldehyd, in as strong a dilution as 1 50 did not inhibit the growth of the organisms studied In my experiments bacteria (*B typhosus, B coli M pyogenes aureus*) grew as readily in a slightly alkaline broth to which hexamethylenamin had been added in the proportion of 1 100 as in normal broth

It is seen, therefore, that hexamethylenamin is of value as an antiseptic only after its conversion into formaldehyd, which must be present in the proportion, at least, of about 1 30,000

TESTS EMPLOYED FOR FORMALDEHYD AND HEXAMETHYLENAMIN

Four different tests were made use of in the examination of material Hehner's test[22] was used to show the presence of hexamethylenamin It is really a test for formaldehyd, but the strong sulphuric acid used in the test quickly converts the hexamethylenamin into formaldehyd, which then reacts, so that it is given equally well by both drugs The amidol[23] and Jorrison's[18] phloroglucin tests were used to detect small amounts of formaldehyd not shown by the Rimini test The amidol test, although very delicate, is not particularly reliable, and is not applicable to all fluids The phloroglucin test is both delicate and reliable Neither test is given by hexamethylenamin Both are qualitative tests only. Rimini's[24] test was modified so as to be used for quantitative estimation[1] of formaldehyd, for which it is reliable up to dilutions of about 1 60,000 It is not given by hexamethylenamin If formaldehyd is present in less amount than 1 60,000, it can be of no practical importance inasmuch as it would not be antiseptic, but the amidol and phloroglucin tests would show even minute quantities (up to 1 10,000,000) With these tests it is possible to determine the presence or absence of both hexamethylenamin and of formaldehyd, to determine whether hexamethylenamin is present alone and if formaldehyd is present, to estimate the quantity up to 1 60,000

EXAMINATION OF NORMAL AND PATHOLOGIC FLUIDS

The material for examination consists of that from eleven cases of cerebrospinal and ventricular fluids, four cases of otitis media, two cases of empyema, one case of pelvic abscess, six normal blood-serums, two cases from which normal bile was obtained, three from which sweat and five from which saliva was collected This material may seem small in amount from which to draw conclusions, but the main object in view was experimental and not clinical Each fluid was subjected to many different tests with particular reference to changes in reaction and the influence of these changes on the liberation of formaldehyd With two exceptions all patients had been given hexamethylenamin by mouth before the fluids were collected In no case was free formaldehyd found All showed the presence of hexamethylenamin After the conversion of this hexamethylenamin with acid there was in no case enough formaldehyd liberated to furnish antisepsis These findings will be outlined briefly and tabulated

Cerebrospinal Fluid.—The spinal or ventricular fluid was obtained by lumbar and ventricular puncture in several cases and tested as shown

22 Hehner Abstr, Lond Chem Soc, 1896, A, ii, 583

23 Referred to in Allen Commercial Organic Chemistry

24 Rimini Chem Zentralbl, 1898, i, 1152, abstr, Journ Soc Chem Industr 1898, 697

TABLE 1.—EXAMINATION OF CEREBROSPINAL FLUID

Case	Diagnosis	Material and Treatment	Tests*			
			Date	Hehner	Rimini	Amidol or Phloroglucin
1	Tuberculous meningitis	Hexam 15 gr., t. i. d., p. c., for several days. Lumbar puncture, 11 c.c. fluid	4/24	+	—	—
		Crystals of acid sodium phosphate added to 3 c.c.	4/24			
		5 c.c. inoculated with *M. pyo. aur.*	4/26	+	—	+
		Incubated at 37 C. for six days	4/24			
			4/30	+	—	+
2	Tuberculous meningitis	Hexam 20 gr., t. i. d., p. c. Lumbar puncture	4/26	+	—	—
3	Lues	Hexam 20 gr., q. 2 h., two days. Lumbar puncture		+	—	—
		Crystals of Na_2HPO_4 added to 5 c.c. after twenty-four hours		+	†	+
4	Medical	Hexam 20 gr., q. h., added 4 doses. Lumbar puncture		+	—	—
		4 c.c. inoculated with *B. coli* after forty-eight hours		+	—	+
5	Medical	Hexam 20 gr., q. h., added 4. Lumbar puncture		+	—	—
6	Hydrocephalus in child	Hexam 5 gr., q. h., added 8. Ventricular puncture		+	—	—
		5 c.c. inoculated with broth culture of meningococcus after fourteen days' incubation		+	—	+
7	Medical	No hexam by mouth. Lumbar puncture		—	—	
		Hexam 1 gr. added to 3 c.c. and tested after standing forty-eight hours		+	—	—
		Crystals of Na_2HPO_4 added to 3 c.c. with hexam 1 gr. and tested after twenty-four hours		+	‡	
8	Hydrocephalus in child	Hexam 40 gr., q. d., for three days. Lumbar puncture		+	—	—
		Ventricular puncture		+	—	
		Above after standing three days		+	—	
9	Hydrocephalus	Hexam 5 gr., t. i. d., p. c., for two weeks. Lumbar puncture		+	—	
		Ventricular puncture		+	—	
10	Hydrocephalus in child	Hexam 5 gr., t. i. d. Ventricular puncture		—	—	—
		8 c.c. made strongly acid with H_2SO_4 after twenty-four hours		—	—	
11	Meningitis	Hexam 40 gr., in day, 75 gr. after midnight. Lumbar puncture at 10 a. m.		+	—	—
		5 c.c. inoculated with *M. pyo. aur.* incubated five days		+	†	

* In this and the following tables, + = positive, —, negative.
† 1 60,000 ‡ 1 20 000

in Table 1 Hexamethylenamin was present in all of the cerebrospinal fluids with the one exception In Case 10 the fluid obtained by lumbar puncture was negative to Hehner's test, and the addition of sulphuric acid failed to render the test positive for formaldehyd In none of these fluids in which hexamethylenamin was present following the administration of the drug by mouth was free formaldehyd found After inoculation of these fluids containing hexamethylenamin with the meningococcus, *M pyogenes aureus* or *B coli*, formaldehyd was not liberated in amounts sufficient to give the Rimini test (1 60,000), although after several days' incubation they were all positive to the delicate amidol or phloroglucin tests for formaldehyd The addition of acid sodium phosphate to the fluid in one case (Case 3) in which the patient had received enormous doses of hexamethylenamin, 20 grains every two hours, caused after twenty-four hours the liberation of formaldehyd in about 1 60,000 dilution Hexamethylenamin added directly to these fresh fluids was not

TABLE 2.—EXAMINATION OF PURULENT DISCHARGES IN OTITIS MEDIA

Case	Material and Treatment	Tests		
		Hehner	Rimini	Amidol or Phloroglucin
1	Hexam., gr. 5, q. 2 h., added doses 6 Discharge obtained by syringing out the ear with small amount of sterile water	—	—	—
2	As above	—	—	—
3	As above	—	—	—
4	As above	—	+	—
5	As above	—	—(?)	—

decomposed, and the subsequent inoculation of these concentrated solutions in no case caused a liberation of formaldehyd in a greater amount than 1 60,000

Purulent Discharge in Otitis Media.—The material was obtained in cases of acute otitis media by syringing out the ear with as small an amount of sterile water as possible With the exception of one case, which gave a positive Hehner's test for hexamethylenamin, all failed to show either hexamethylenamin or formaldehyd The findings are not accurate, inasmuch as the discharges were necessarily diluted before being tested, but the failure to find even minute quantities of either drug are significant The findings are grouped in Table 2

Pus of Empyema.—Two patients with empyema were given hexamethylenamin for a few days, and at operation the pus was obtained and examined as is shown in Table 3 All tests were uniformly negative,

showing the absence of both hexamethylenamin and formaldehyd, but the pus was strongly acid in reaction, and when hexamethylenamin was added to it *in vitro* it was quickly decomposed into formaldehyd

Pelvic Abscess and Pus-Tube.—One patient was given hexamethylenamin, 20 grains, after each meal for two days before an operation for pus-tubes and a pelvic abscess. The pus obtained at operation was examined with negative results for all tests. The reaction of the pus was strongly acid, 9.7 c.c. tenth-normal sodium hydroxid to 10 c.c., and when examined four hours after the addition of 5 grains of hexamethylenamin to 40 c.c. of it formaldehyd was present in the proportion of 1:10,000.

Blood-Serum.—Venous puncture was done in six cases in which the patients were being given a routine dosage of 15 grains of hexamethylenamin after each meal, and the serum after separation was tested with negative results in all cases for formaldehyd, but positive in all for hexamethylenamin. The addition of acid sodium phosphate to these serums in no case caused a liberation of formaldehyd in sufficient amount to give a 1:60,000 test, although the more delicate formaldehyd tests were uniformly present after this treatment.

TABLE 3.—Examination of Pus of Empyema

Case	Material and Treatment	Reaction	Test		
			Hehner	Rimini	Amidol or Phloroglucin
1	Hexam., 10 gr., t. i. d., p. c. Operation after two days and 600 c.c. of pus obtained which showed a pure culture of pneumococcus	13.6 c.c. Tenth-normal sodium hydroxid to 10 c.c.	—	—	—
	Hexam. added to 200 c.c. of above examined after twenty-four hours		+		+
	(Culture made from this on the seventh day was negative) after seven days	After 7 days	—	†	
2	Hexam., 15 gr., t. i. d., p. c. Operation three days, 300 c.c. pus obtained	16 c.c.	—	—	—

* 1:10,000. † 1:7,000.

Sweat.—Two patients receiving hexamethylenamin, 15 grains, after each meal, were placed in a sweat-cabinet, their perspiration collected[25] and examined with positive results in both cases for hexamethylenamin. The Rimini test was negative in both. Unfortunately, the more delicate

25 Through the kindness of Dr. Harry Plaggemeyer.

phloroglucin test was not tried in these cases Sweat is slightly acid in reaction, and possibly the small amount of hexamethylenamin present had been converted into formaldehyd, which would also give the delicate Hehner's test It was not, however, present in anything like an antiseptic strength

Saliva—The saliva was collected from five patients receiving a routine dosage of 15 grains after each meal A positive Hehner's test was obtained in all but one case, but the other tests were all uniformly negative

Bile—Hexamethylenamin when taken by mouth is excreted in the bile Free formaldehyd, however, is not found After the addition of hexamethylenamin to sterile bile *in vitro* even in the proportion of 1 100, it is not decomposed so long as the bile is kept sterile Infection of these concentration bile solutions causes a liberation of formaldehyd, but in the two cases tested not more than 1 60,000 was found There has been no opportunity to examine the contents of the gall-bladder in cases of infection after feeding hexamethylenamin It is possible that in cholecystitis or empyema of the gall-bladder its contents may have a sufficient acidity readily to convert the hexamethylenamin excreted into it

Hexamethylenamin in Standard Broth—In order further to test the influence of infection on the reaction of fluids and on the subsequent liberation of formaldehyd, known dilutions of hexamethylenamin were made up in standard broth, inoculated with various organisms and tested after certain periods for changes in reaction and for free formaldehyd The findings are given in Table 4 The changes in reaction are slight in all cases, so much so that in no case was more than 1. 60,000 formaldehyd found after the growth of an organism in a 1 1,000 hexamethylenamin solution for as long as five days, and the growth of the organism was in no case inhibited

SUMMARY OF EXAMINATION OF NORMAL AND PATHOLOGIC FLUIDS

The amount of decomposition of hexamethylenamin into formaldehyd is so clearly dependent on acidity and concentration that the problem resolves itself into a determination of these factors None of these fluids are normally acid, and whatever tendency there is for them to become acid is being continually counteracted by the alkaline circulation On the other hand, the amount of hexamethylenamin present in these fluids has been found to be relatively small, which is no doubt due to the fact that it is rapidly eliminated by the kidneys and is not given an opportunity to accumulate Formaldehyd, if it is liberated within the body, is

TABLE 4.—Ilsis of Hexamethylenamin in Standard Broth

Organism	Dilution	24 Hours			48 Hours			72 Hours			120 Hours		
		Reaction*	Formaldehyd†		Reaction*	Formaldehyd		Reaction*	Formaldehyd		Reaction*	Formaldehyd	
			Rimini	Amidol		Rimini	Amidol		Rimini	Amidol		Rimini	Amidol
B coli	1 1,000	Alk 1	—	+	Acid 2	—	+	Acid 4	+	+	Acid 4	+	+
	1 10,000	Alk 1	—	+	Acid 4	—	+	Acid 6	—	+	Acid 6	—	+
B typhosus	1 1,000	Acid 4	‡	+	Acid 6	+	+	Acid 6	+	+	Acid 6	+	+
	1 10,000	Acid 2	—	+	Acid 4	—	+	Acid 6	—	+	Acid 6	—	+
B proteus vulgaris	1 1,000	Acid 1	—	+	Acid 4	—	+	Acid 4	+	+	Acid 6	+	+
	1 10,000	Acid 2	—	+	Acid 4	—	+	Acid 6	—	+	Acid 6	—	+
Streptococcus pyogenes aureus	1 1,000	Alk	—	—	Alk	—	—	Acid 2	—	+	Acid 8		+
	1 10,000												
Meningococcus	1 1,000	Alk	—	—	Acid 2	—	+	Acid 4	—	+	Acid 4	—	+
Alkaligenes	1 1,000	Alk	—	—	Alk	—	—	Alk	—	—	Alk	—	—
Control typhoid	0	Alk	—	—	Acid 2	—	—	Acid 4	—	—	Acid 8	—	—
Control hexamethylenamin	1 1,000	Alk	—	—	Alk	—	—	Alk	—	—	Alk	—	—
Control urine	1 1,000	Acid	¶	+	Acid 2 6 §	**	+	Acid 2 6 §	*	+			

* The numerals under "Alk" or "Acid" indicate the number of drops of tenth-normal hydrochloric acid (alk.) or tenth-normal sodium hydroxid (acid) necessary, respectively, to neutralize 10 c.c. of the solution with phenolphthalein as indicator

† In the tests, + means positive and — negative

‡ Percentage of formaldehyd, 1 60,000

§ The figures in the lowest column under "Acid" indicate the number of cubic centimeters of tenth normal sodium hydroxid needed to neutralize 10 c.c. of urine

20 000 ** Percentage of formaldehyd, 1 10,000

an irritant, and as such is not tolerated by the organism Maguire[26] found that intravenous injection of formaldehyd into animals produced gastric irritation with ulcer formation, hematuria and hemoglobinuria and death Several of my patients were fed liquor formaldehydi (in dilution of 1 3,000, 6 ounces every three hours), and afterward their blood and urine were repeatedly found to be negative to all tests for both hexamethylenamin and formaldehyd, showing that the body can in some way burn up these small amounts The fate of the formaldehyd which gets into the circulation is uncertain There is experimental evidence to suggest that when not excreted by the kidneys it is neutralized into a harmless polymer and possibly hydrolyzed in the liver into one of the sugars The conditions, therefore, for the decomposition of hexamethylenamin in antiseptic amounts are seldom, if ever, realized in these alkaline body-fluids

From these facts and from the uniform negative findings on direct examination it is evident that hexamethylenamin can be of no value in the alkaline or neutral fluids of the body, such as found on the serous surfaces in general This will be true even when enormous doses of the drug are administered, which is shown by no conversion occurring in these fluids when large amounts of the drug are added directly to them Hexamethylenamin, therefore, can be of no prophylactic benefit in any of the body-fluids except those that are normally acid, and the only three fluids of this character are the urine, sweat and gastric juice

The question of the influence of infection on these alkaline fluids relative to the liberation of formaldehyd is more difficult to answer The examinations for formaldehyd of the pathologic fluids of patients given hexamethylenamin by mouth were all uniformly negative After the subsequent addition of hexamethylenamin to these fluids, with the exception of localized accumulations of pus, no decomposition of this into formaldehyd was found In local accumulations of pus, as in empyema or abscesses, this may have a pronounced acidity, and the addition of hexamethylenamin even in amounts of 1 10,000, to this pus quickly liberates formaldehyd *In vivo,* however, these pus-pockets are apparently so well walled off that very little of the hexamethylenamin in the blood finds its way into them If hexamethylenamin did enter in at these local areas, the formaldehyd formed there would naturally have as ready access to the circulation and would be carried off as fast as formed, so that no accumulation for antiseptic purposes would occur The gall-bladder, in those infections in which its contents become acid, may be a possible exception

The subsequent inoculation with organisms of fluids obtained from patients taking hexamethylenamin by mouth, even on seven days' incu-

26 Maguire Med News, 1903, lxxxiii, 393

bation, in no case liberated formaldehyd in antiseptic amounts, although the more delicate tests of those showing formaldehyd up to 1 10,000,000 were sometimes obtained And after the addition of as much as 1 1,000 hexamethylenamin to these fluids and then their inoculation, formaldehyd was liberated only after several days in 1 60,000 amounts The inoculation of broth solutions of hexamethylenamin in no case showed greater conversion than 1 60,000, and then only after seventy-two hours Infection of these fluids *in vitro,* therefore, even under the most favorable circumstances, that is, when hexamethylenamin has been added in as high a dilution as 1 1,000, a concentration that would never obtain *in vivo,* does not produce sufficient change in reaction to form formaldehyd in antiseptic strength Furthermore, those fluids containing hexamethylenamin after its ingestion by mouth, when made strongly acid with acid sodium phosphate or sulphuric acid, did not contain hexamethylenamin in amounts to liberate more than 1 60,000 formaldehyd, showing that hexamethylenamin was not present in sufficient concentration to furnish antisepsis even should all present be decomposed

It is reasonable to assume, therefore, that infection in the body does not change the reaction of the body-fluids into which hexamethylenamin is secreted sufficiently to cause its conversion in antiseptic amounts In case of localized accumulations of pus of marked acidity, hexamethylenamin does not gain access in amounts to be of value, and in the case of other infection in communication with the circulation, the small amount of formaldehyd formed is quickly taken up and metabolized or excreted by the kidneys

CONCLUSIONS

Hexamethylenamin is dependent on the liberation of formaldehyd for its antiseptic value

Hexamethylenamin is not converted into formaldehyd in any of the normal alkaline fluids of the body, therefore, it can be of no prophylactic value in any of these fluids

After some infections of these fluids there may be under certain conditions a change in reaction sufficient to produce slight liberation of formaldehyd, but it was not possible to show that there would be enough to give antisepsis

In localized infections of pronounced acidity, hexamethylenamin is not taken into them from the circulation in amounts to furnish formaldehyd in antiseptic strength (the gall-bladder, possibly, excepted)

The therapeutic use of hexamethylenamin as an internal antiseptic is justified, experimentally, for urinary conditions alone, and then only when it is excreted into an acid urine

HEXAMETHYLENAMIN *

HUGH McGUIGAN, M D, AND C L v HESS, M D
CHICAGO

Hexamethylenamin is widely used as an antiseptic to prevent and to combat various infections of the serous cavities and blood as well as those of the urinary tract, the wide range of application being due to the striking effects obtained in some cases of cystitis and allied conditions, as well as to the great penetrability of the drug, which after ingestion can be found in every fluid of the body [1] These facts, as well as the possible liberation of free formaldehyd, to which the efficiency of the drug is usually ascribed, would seem to make it an ideal drug in infections, and great claims have been made for its efficacy But the question arises Is formaldehyd liberated, and if so, is it in sufficient amount to have an antiseptic action?

Hexamethylenamin is readily decomposed into formaldehyd and ammonia in acid solutions, less readily in neutral or slightly alkaline solutions, and not at all in a stronger alkaline medium When injected intravenously it produces a temporary increase in the rate and depth of respiration which would indicate an ammonium action and a consequent formaldehyd liberation In addition, formaldehyd is always found in acid urines after administration of the drug These results might mean a liberation of formaldehyd in all parts of the body, but Hanzlik and Collins[2] and others have failed to find such a liberation in any of the body fluids except the gastric juice and urine There is a possibility, however, that it is liberated and yet not detected, since one of us (McGuigan) has shown that small amounts of formaldehyd are oxidized in the blood so rapidly that it quickly escapes detection by the ordinary means, even that introduced into the stomach or liberated from hexamethylenamin by the gastric juice and subsequently absorbed To meet this objection a new method is offered for the detection of free formaldehyd in the blood This consists in dialyzing the normal circulating blood in animals through collodion tubes interposed between the carotid

* From the Laboratory of Pharmacology of the Northwestern University Medical School

* Submitted for publication Dec 29, 1913

1 McGuigan, Hugh Jour Biol Chem, 1912, xi, 33, proceedings

2 Hanzlik, Paul J, and Collins, R J Hexamethylenamin The Liberation of Formaldehyd and the Antiseptic Efficiency Under Different Chemical and Biological Conditions, THE ARCHIVES INT MED, 1913, xii, 578 This gives a complete review of the literature

artery and the external jugular vein [3] The tubes are immersed in water or saline solution and the living blood is dialyzed during the action of the drug If any formaldehyd is formed, part of it will rapidly pass into the dialysate, where it can be identified by the usual tests The results show uniformly that hexamethylenamin is found in the dialysate in a few minutes after the injection, while free formaldehyd is not detected even after several hours of dialysis The following protocol will illustrate

Male dog Ten kg Ether anesthesia

1 Collodion dialyzing tubes attached to the carotid artery and external jugular vein

1 20 Dialysis commenced Tests of the dialysate for hexamethylenamin and formaldehyd negative 100 c c of 3 per cent hexamethylenamin in 0 9 sodium chlorid slowly injected into the femoral vein in the next ten minutes

1 40 Dialysate tests Hexamethylenamin positive, formaldehyd negative

2 Dialysate tests Hexamethylenamin positive, formaldehyd negative

4 10 Dialysate tests Hexamethylenamin positive, formaldehyd negative Urine strongly positive to free formaldehyd tests

4 30 During the next ten minutes 40 c c 0 5 per cent formaldehyd injected slowly into the femoral vein

4 40 Dialysate test shows free formaldehyd

In control experiments the injection of formaldehyd only always gave free formaldehyd in the dialysate Some hexamethylenamin is also formed The Jorrisen and Rimini tests were used for free formaldehyd, and Hehner's test for the combined form

It is evident that formaldehyd is not liberated in amounts sufficient for detection with the phloroglucin or phenylhydrazin tests Since these reagents will detect amounts far less than enough to have any antiseptic action, hexamethylenamin antisepsis, if any, cannot be due to decomposition products But even though formaldehyd were liberated in minute amounts it would not have the reputed hexamethylenamin effect, since after injection small amounts of free formaldehyd are so rapidly oxidized that antiseptic action is improbable, while in larger amounts it is decidedly injurious

Hexamethylenamin itself has no antiseptic action on yeast or bacteria outside of the body, but the possibility still remains that the mere entrance and exit of the drug may have a beneficial action when taken internally

3 Abel, John J , Rowntree, Leonard G , and Turner, B B Removal of Diffusible Substances from the Circulating Blood of Living Animals by Dialysis, Jour Pharmacol and Exper Therap , 1914, v, 275 This gives Abel's method without a coagulant We were unaware of the priority of Abel in this field when the work was done

SUMMARY

Since, after the administration of hexamethylenamin, free formaldehyd appears only in the gastric juice and acid urine, and not in other secretions or in the blood, even with dialyzing tests, the liberation results from the acid reaction only and not from cell action Even if it were liberated in other locations it would probably exert no beneficial action because of its reconversion or rapid oxidation into injurious products. Combined formaldehyd as hexamethylenamin, does not decompose readily until an acid medium is reached, when the action is that of weak formaldehyd The only apparent indication for the internal administration of the drug, therefore, is in infections of the urinary tract

SOME RELATIONS OF THE BRAIN AND OF THE OLFACTORY APPARATUS TO THE PROCESSES OF IMMUNITY*

HENRY SEWALL, M D
DENVER

The body is provided with three obvious lines of defense against infection, namely, its peripheral epithelium, its blood and lymph, and its tissue cells The activity of modern investigators has been engrossed almost wholly with study of the last two, the first has received very little attention Yet it is indisputable that the epithelial covering of the body must have intrinsic resources of protection against adverse influences which are in such habitual action that its relations should be considered truly physiological, rather than pathological, as its response to adventitious agencies would be In short, a study of the pathologic physiology of the peripheral epithelium must conceive of it primarily as a mechanism for the *prevention* of disease rather than, as in the case of the body-fluids and tissues, an establishment for producing the means of *cure* While the protoplasm of the surface cells must be endowed with a degree of autonomy involving capacity for independent response to irritation, which possibly includes the production of specific antibodies, its striking peculiarity is its abundant supply of sensory nerves, largely endowed with "specific energy," through which every external stimulus is made to provoke an immediate and universal internal response

The incitement to the investigation the report of which follows was a desire to develop the existence of some immunological relations between the peripheral and the central mechanisms If *a priori* conceptions are given undue prominence at the outset, a measure of defense may be found in the wise dictum of Richet,[1] that in biologic research the guiding thread must always start from the teleologic point of view

Modern therapeutics offers abundant illustration of the beneficent influence of physical agencies acting externally The criterion for the usefulness of cold water in the treatment of typhoid fever is the "physiological response" which its application may provoke As Chantemesse points out, the hydriatric stimulus probably does not excite the formation of specific antibodies against the invading noxa, but it seems probable that the *capacity* for the formation of antibodies is enhanced indi-

* Submitted for publication Dec 17, 1913

1 Richet, Charles Ann de l'Inst Pasteur, 1907, xxi, 497

rectly, through reflex excitement of the various motor and metabolic mechanisms Similarly, the benefits of the open-air treatment in tuberculosis are in some way due to physiological response to external agents, which, as in another place I have attempted to show, exert their influence largely through the temperature nerves of the skin There is good reason to believe that at least the vital capacity for the formation of antibodies is largely under the control of external stimuli which make up the environment of what is known as the "hygienic life" The fact that tuberculosis finds least resistance to its development in the tissues of those who, like the American Indians, from a habitual open-air life are suddenly forced to conform to the restraints of civilization, argues that hygienic living produces no specific antibodies against this disease

But there is abundant clinical evidence that a great variety of antigens can produce specific anaphylactic systemic response when applied to the peripheral epithelium The assumption that such antigens must be absorbed into the circulation before becoming effective, while probably correct in general, is unwarranted without direct evidence The urticarias and other anaphylactic phenomena following the ingestion of certain articles of food by some people, and, especially, the asthmatic paroxysms induced by the inhalation of certain odors, belong to one of the most interesting divisions of clinical pathology Indeed, it was an incident related to me by V C Vaughan, Jr, of Detroit, which gave the final impulse to the present investigation

Vaughan's account is as follows The patient was a man, aged 36, who was suffering from moderately advanced tuberculous disease of the lungs complicated by recurrent hemoptyses From March 11 to 13, inclusive, the patient received three injections of fresh, normal rabbit's serum of 0 1 c c, 9 c c and 10 c c April 7, twenty-seven days after the first injection, a rabbit was brought into the patient's room, was handled by him and allowed to run about the bed Between three and five hours after this event the patient suffered an asthma-like attack — the first which he had ever experienced

As indicated at the outset, my conception in the main has been that phenomena which are essentially anaphylactic form a conspicuous feature of normal sensory activity The mysterious ability of the hound to follow a definite trail unconfused by a multitude of other footsteps is cleared up at once if we assume that the dog's olfactory apparatus is sensitized to the odor which he follows One is tempted to suggest that even the phenomena of complementary color sensation, as manifested in the "after-images," are but the psychic signs of specific antibodies formed in the retina

The experiments to be described were performed on guinea-pigs Sensitization was achieved by subcutaneous injection of normal horse-

serum obtained from a commercial source The sensitizing dose usually varied from 0 25 to 0 5 c c The toxic injection was invariably given through the jugular vein Many intracranial operations were performed, including cutting the olfactory nerves or ablation of the olfactory lobes alone or with a certain bulk of the anterior cerebrum Anatomically the guinea-pig is excellently adapted for such work As is well known, the anatomical relations of the sense-organ for smell are almost unique [2] It is constructed peripherally of nerve-cells inclosed within the olfactory mucous membrane Processes directed distally from the nerve-cells project freely like bristles from the free surface of the membrane, while from the opposite poles "teledendrons" find their way into the cranial cavity to embrace the nerve-cells of the olfactory lobes

This research would have been quite impossible but for the devoted aid given by my colleague, Dr Cuthbert Powell, who has freely lent his surgical skill to the performance of the many scores of surgical operations involved in the investigation

GUINEA-PIGS SENSITIZED TO HORSE-SERUM MAY BE DESENSITIZED BY SOJOURN IN A HORSE STABLE

The first effort made was to elicit some sign of anaphylaxis in the peripheral epithelium of a serum-sensitized animal A guinea-pig, which had received a subcutaneous injection of horse-serum thirty-eight days before, was held gently on its back and horse-serum in droplets was allowed to fall into each nostril I expected to witness some sign of specific irritation The result, however, was wholly negative The animal a few hours later received an intravenous injection of 0 5 c c of horse-serum and died in about three minutes with characteristic symptoms Another sensitized pig was made to inhale finely vaporized serum, in the manner to be described later, but, as in the first case, the results were negative These observations were repeated on numerous occasions with different animals and the results all pointed to the conclusion that *guinea-pigs sensitized by subcutaneous injections of horse-serum manifest no particular irritation when the same serum is instilled into the nose or given by inhalation*

It then occurred to me that possibly the aroma of the animal producing the sensitizing antigen might develop some characteristic reaction Accordingly, four guinea-pigs were placed in a box covered with wire netting Two of the animals were normal and two, forty days previously, had each received subcutaneously from 0 5 to 0 75 c c of serum The box was carried, July 7, to a livery stable in which a score or more of horses were maintained The air of the stable at this time was powerfully impregnated with a characteristic odor and there

2 Troland Am Jour Physiol, 1913, xxxii, 19

was a particularly pungent quality to the smell Later in the season, as cooler weather advanced, the intensity of the odor diminished and its pungent quality disappeared There was, as will be seen directly a coincident change in the biological reactions The box had been in the stable possibly five minutes when marked nervous disturbances were manifested by the two sensitized animals One of them, a female weighing 480 gm , showed marked excitement, dashing about the box and biting at her companions, then scratching the nose vigorously These symptoms subsided in about half an hour The other sensitized pig, weight 195 gm , lay prone or squatted on the floor of the box and emitted plaintive sounds The animals were kept in the stable overnight and appeared wholly normal when removed next morning The control animals showed no particular disturbance

Some drops of serum were instilled into the nostrils of the smaller sensitized pig and provoked within a few moments somewhat the same reactions as the animal had manifested in the stable This experiment was, under these conditions, unfortunately not repeated More than twenty-four hours after its introduction into the stable and about eight hours after its removal therefrom, the larger sensitized pig was given by the vein nearly 1 c c of horse-serum *There was no marked reaction,* soon after its release from the holder the animal fed as usual Twenty-five days later this pig received another intravenous injection of serum and died within four minutes The smaller of the two sensitized pigs received its toxic dose some twenty-eight hours after removal from the stable Owing to difficulties in manipulation a great excess of serum was injected into the vein, probably more than 1 c c The animal did not suffer the usual shock, but died from respiratory failure in about half an hour,' its lungs were almost completely collapsed when the chest was opened

In the light of later observations I am inclined to think that this animal's death was a sign of the passing of the phase of antianaphylaxis induced by inhalation of stable-air combined with the excessive magnitude of the toxic injection No effort was spared, of course, in seeking a solution of the results obtained in these preliminary experiments Of fundamental importance appeared to be the question whether the desensitizing agent in the air of the stable affected the body indirectly through action on the olfactory mucous membrane or was absorbed directly through the respiratory tract To resolve this question, groups of pigs, sensitized or normal, were operated on for section of the olfactory nerves Under ether, a small hole was trephined just posterior to the cribriform plate, the nerves were cut with a fine bistoury and their stumps wiped out with a small pledget of cotton held in forceps The animal manifests little or no disturbance after such an operation

In other animals not only the olfactory nerves were removed but the olfactory lobes as well But, for reasons that will be detailed later, this more extensive operation was necessarily performed only on normal, non-sensitized animals The experimental data are condensed in the following protocols

EXPERIMENT 1.—Four sensitized guinea-pigs, two with intact olfactories, exposed to stable August 18

Pig 1, male, 480 gm , olfactory nerves excised July 12, sensitized with serum July 16 No reaction in stable

Pig 2, male, 645 gm , sensitized July 16, olfactory nerves excised August 15 Restlessness after twenty minutes in stable

Pig 3, male, 675 gm , unoperated, sensitized July 16 Much erotic excitement after fifteen minutes in stable

Pig 4, male, 750 gm , unoperated, sensitized July 16 No reaction except slight uneasiness after forty minutes in the stable

After remaining all night in the stable each animal received. August 19, an intravenous injection of more than 0 5 c c of fresh serum Every pig died in characteristic shock except Pig 3, which responded to the injection with violent struggles, some respiratory râles, deepened respiration and rubbing of the nose when released After this moderate shock the pig completely recovered

One cannot fail to connect the excitement of this pig when exposed to the stable air, attempting to copulate with his male companion, etc , with the desensitization certified by the development of antianaphylaxis This animal was reexposed in the stable thirty-three days later, when he showed but slight excitement, and the following day he succumbed to intravenous injection

EXPERIMENT 2.—Two pigs received subcutaneous serum July 16 Exposed in stable August 20

Pig 1, male, 525 gm Olfactories intact Within five minutes after entering stable is erotic and continues restless, scratches nose, etc , for an hour

Pig 2, male, 540 gm Olfactory nerves had been cut July 12, and a serum inhalation given August 16 In the stable is restless, slightly dyspneic and scratches chin during an hour

August 21, intravenous injection of serum, more than 0 5 c c , was given to each Pig 1 had two slight spasms, sneezed twice and then recovered Pig 2 showed very little reaction Result, antianaphylaxis in both animals

The recovery of the second animal would appear to show that the olfactory apparatus was not concerned in the desensitization, but on August 22, its brain was exposed for the purpose of excising the olfactory lobes, it was then found that the olfactory nerves appeared to have regenerated in the interval of thirty-nine days elapsing between the nerve section and the stable exposure

EXPERIMENT 3.—Two pigs sensitized July 16. Both had been in the same box with the animals used in a former experiment. They were placed again in the stable for forty minutes, August 22. Twenty hours later they received the toxic injection.

Pig 1, male, 660 gm. Somewhat erotic and restless in stable.

Pig 2, male, 360 gm. Olfactory nerves cut July 12. Restless and rubs nose in stable.

Both died from the toxic injection, which, it will be noted, was given twenty hours after leaving the stable, whereas the usual interval was two or three hours. Necropsy on Pig 2 indicated partial regeneration of the olfactory nerves.

EXPERIMENT 4.—Two pigs sensitized July 16. Placed in stable for five hours, August 25.

Pig 1, 425 gm., a comparatively feeble animal. Restless, rubs nose and scratches in the stable.

Pig 2, 790 gm., olfactory nerves were cut, August 24. Less excitement shown in the stable than in the case of Pig 1.

Intravenous injections of more than 0.5 c.c. of serum were given within an hour of removal from the stable. Pig 2 died promptly from shock. Pig 1 showed no reaction except for temporary muscular weakness becoming apparent in an hour. This animal was undoubtedly antianaphylactic but was found dying the next morning after exposure to the cold of the previous night. It was frequently noted that guinea-pigs recovering from the strain of anaphylactic shock are exceedingly non-resistant to external cold. Their temperature regulation is apparently profoundly disturbed.[3]

It is unnecessary to go further into detail along this line of evidence. Suffice it to say that a large proportion of guinea-pigs sensitized subcutaneously to horse-serum were desensitized by exposure to the air of the horse-stable. There was a very obvious relation between the pungency of the stable-odor and the latent period of anaphylactic disturbance. When the smell was most intense the nervous symptoms of anaphylaxis came on almost immediately. In Denver the nights became strikingly cool in August so that the more intense stable-odor only endured for two or three weeks in July and the time required to sensitize guinea-pigs did not allow of prolonged investigation under conditions most favorable to olfactory stimulation.

As the mean temperature of the air fell and the stable odor became less intense the latent period of anaphylactic disturbance increased from a very few minutes to a quarter of an hour, then to longer periods and finally the onset of disturbance could not be distinguished. Nevertheless sensitized pigs kept in the stable for a day or more sometimes

3 Doerr. In Kolle and Wassermann's Handbuch des pathogenen Mikroorganismen, 1913, II, Part 2, p. 1070.

proved to be antianaphylactic to intravenous injection even when they had manifested no striking symptoms marking desensitization

All the earlier observations pointed to the conclusion that only pigs possessing intact olfactory mechanisms were subject to desensitization through the stable air Obviously a single positive result demonstrating antianaphylaxis in the case of a smell-free guinea-pig would show that the noxa producing desensitization could affect the body through channels other than the olfactory Such a demonstration was afforded by observations on four animals kept in the stable for three days during the first week in October Each had suffered either recent section of its olfactory nerves or loss of both olfactory nerves and lobes many weeks previously Submitted to the test of intravenous inoculation, one of the pigs whose olfactory lobes had been excised thirty-nine days before and which had been sensitized by subcutaneous serum thirty-six days before, recovered from the toxic injection after slight symptoms of shock

This section of my work may be briefly summarized by stating that fourteen sensitized guinea-pigs with intact olfactory sense were exposed to the stable air Of these, five became completely antianaphylactic to the intravenous toxic injection and two succumbed only after evidence of great resistance to the toxic dose The fourteen pigs included two that had been previously made antianaphylactic as witnessed by recovery from an intravenous injection, both died from the second intravenous dose The fourteen pigs also included two in which the olfactory nerves had been cut long before but which seemed to have regenerated

Ten sensitized pigs were exposed in the stable after recent section of their olfactory nerves or long-standing ablation of the olfactory lobes None of these had manifested any marked symptoms of anaphylactic disturbance in the stable Only one of this group survived the toxic intravenous injection

Daily contact with such phenomena as have been described is bound to give rise to certain more or less defensible impressions which have not been submitted to definite proof Of this nature are the notions that the desensitizing agent in the stable-air operates on the body in two ways (1) acutely, as an odor irritating the olfactory nerves with the production of anaphylactic symptoms, (2) more slowly, by absorption through the respiratory tract, without the production of striking anaphylactic symptoms The important question as to whether antianaphylaxis can be produced by the mere odorous stimulation of the olfactory epithelium has not been settled, although the facts seem to point in that direction The phase of antianaphylaxis in these experiments, as found in other fields, comes on very soon after exposure to the exciting cause Also, several experiments pointed to the conclusion

that the antianaphylactic period is very short — that it is rapidly vanishing within twenty-four hours after removal from the desensitizing environment

The theory having been proved that fully developed sensitization could be temporarily undone by exposure of animals to stable air, the conclusion seemed obvious that sensitization should be altogether prevented in pigs kept in the stable during the latent period of sensitization The result of an appropriate experiment proved, however, directly the contrary, as follows

EXPERIMENT 4 —Five guinea-pigs, weighing about 350 gm each, were placed in the stable October 24, about twenty hours after receiving subcutaneous injections of serum, with the exception of one animal which had received an injection ten days before This last pig was taken from the stable October 29, that is, fifteen days after its sensitizing injection and at the end of five days' residence in the stable An intravenous injection given at this time was fatal within four minutes The remaining four pigs were taken from the stable November 17, after exposure of fourteen days, and each succumbed to an intravenous injection within about three minutes Necropsy was performed on these, as in all other fatal cases, and the lungs were found in characteristic anaphylactic distention Guinea-pigs of this group manifested a somewhat striking difference in behavior from all other animals exposed in the stable They never recovered from the timidity associated with the change of environment, nor did they acquire a normal appetite for food The results of the experiment indicate that, at least in cool weather, the processes leading to sensitization go on in the environment which may effect desensitization of a fully sensitized animal

SENSITIZATION TO HORSE-SERUM IS NOT CAUSED BY EXPOSURE TO STABLE AIR

As to the nature of the agent producing the desensitizing effects, I have no definite evidence When the box containing the guinea-pigs was held directly under the nose of a horse, or when the manure-impregnated straw of the stable was placed within it no particular reaction was observed An effort was made, of course, to sensitize the guinea-pigs to horse-serum by the same influence which caused their desensitization

The two normal pigs which had been used as controls and exposed to stable air, July 7, each received an intravenous injection of 0 5 c c of horse-serum sixty-three days later There was nothing like shock to be discerned, though one of the animals rubbed its nose vigorously ten minutes after its release, the other seemed sleepy and both preened the body as if stimulated by itching

Four normal male pigs, weighing about 390 gm each, were placed in the stable, September 9 One of them was removed after twenty-four hours and within an hour thereafter received an intravenous injection There was no reaction except that the animal remained rather unusually quiet For fourteen days the remaining pigs were kept in the stable,

two of them being taken out for some hours each day One of these and the third pig — which had been continuously in the stable — were given intravenous injections within about an hour after removal, with completely negative results The evidence shows that while the possibility of minor degrees of sensitization to toxic injections of serum cannot be denied, nothing like the sensibility leading to fatal shock is induced [4] The slighter degrees of anaphylactic reaction are somewhat uncertain in significance since the normal pig is prone to manifest the same actions, such as nose-rubbing and preening the body.

ANAPHYLACTIC SHOCK AND DESENSITIZATION CAUSED BY OPERATIONS ON THE BRAIN OF THE SENSITIZED GUINEA-PIG

According to the well-known views of Besredka,[5] sensitization in the guinea-pig is primarily or wholly a property of the brain This author holds the view that during the process of sensitization the body tissues form a substance *une sensibilisine,* which fixes itself to the brain-cells Intracerebral injection of a large dose of serum (0 25 c c) produces rapid desensitization with great shock or death With small intracerebral injections (0 0025 c c to 0 025 c c), desensitization of the cerebrum comes gradually and the animal becomes antianaphylactic The animal is resensitized by a new injection made within this period He holds that the condition of antianaphylaxis is a return to the normal state Guinea-pigs may be desensitized by intracerebral injections but cannot be sensitized thereby, that is, the brain-cells cannot prepare the sensitizing substance In an animal thoroughly narcotized by ether, the intracerebral injection of serum produces no shock but still induces antianaphylaxis

That brain sensitization cannot altogether account for the phenomena of anaphylactic shock was sufficiently demonstrated by Auer and Lewis,[6] who found that sensitized guinea-pigs were killed by intravenous injection and showed characteristic distention of the lungs after section of the vagi nerves and after destruction of the base of the brain, medulla and spinal cord That the irritability of the brain is profoundly modified by the state of sensitization was early made clear in our experiments

In attempting to remove the olfactory sense from sensitized pigs in preparation for the stable experiments, it was found that while intracranial section of the olfactory nerves produced no particular reaction,

4 Not until this work had been long completed did I learn that Rosenau, in an announcement I have not been able to trace, describes successful efforts to sensitize guinea-pigs in a horse stable

5 Besredka, A, and Steinhardt, E Ann de l'Inst Pasteur, 1907, xxi, 117, Besredka, A Ann de l'Inst Pasteur, 1907, xxi, 777 and 950, ibid, 1910, xxiv, 273, 879, etc

6 Auer and Lewis Jour Exper Med, 1910, xii, 151

ablation of the olfactory lobes caused profound disturbance, as illustrated in the following experiment

EXPERIMENT 5.—Four guinea-pigs, about 730 gm. each, were given subcutaneous injections of 0.33 c.c. of serum, July 16. August 15, under light ether anesthesia, the olfactory nerves were cut and wiped out in three animals, there was no special reaction. In the fourth pig, which received the least ether, both olfactory lobes and nerves were removed. After its release the animal continued to lie on its side and in about fifteen minutes severe clonic convulsions of the limbs appeared, lasting half an hour or more. The animal gradually recovered, but more than two hours after the operation manifested rhythmic "bucking" movements of the body without the feet leaving the floor. Next day this animal was well and was given 0.75 c.c. of serum intravenously, there was no reaction except for slight torpor. One of the first three pigs was given a like injection immediately afterward and died within three minutes.

It should be said that removal of the olfactory lobes in a normal guinea-pig produces no definite response. The shock and subsequent antianaphylaxis described are no special effect of removal of the olfactory lobes, but follow mutilations of the cerebrum, thus

EXPERIMENT 6.—A vigorous male pig, 700 gm., was sensitized July 16. August 23, under ether, an amount of cerebral tissue in bulk about that of the olfactory lobes was removed from the parietal regions. The animal when released continued for ten minutes to lie on its side with clonic twitching of the hind legs. The next day this pig received an intravenous injection of 0.75 c.c. of serum and, with the exception of slight asthmatic wheezing, showed no special reaction.

EXPERIMENT 7.—Male pig, 780 gm., sensitized August 14. August 21, a bulk of cerebrum in size five or six times that of the olfactory lobes, was removed. For nearly an hour the pig lay on its side with clonic twitchings, increasing to convulsions, of the legs. It sprang up and bucked after sitting up and then for many hours carried on continuous coordinated forced movements, seeking always to escape restraint and wander forward. The next morning the animal was found dead. The fatal result was probably brought about partly by failure to react to the lowered temperature of the night air.

Numerous experiments of like character have definitely proved that anaphylactic shock is caused by mutilation of the brain in the sensitized guinea-pig. The degree of shock seems rather strictly related to the amount of cerebral tissue removed, but varies somewhat in different animals. The intensity of shock suffered after a definite nerve injury seemed to diminish as the weather became cooler. If this impression is confirmed it may be elucidated by the facts portrayed in the next section of this paper. In the accounts[7] of anaphylactic shock and the resulting phase of antianaphylaxis usually given, the phenomena have been induced by reinjection of the antigen which caused sensitization, but here are examples of profound somatic changes being induced by mere ablation of brain-tissue in amounts whose loss would cause no particular disturbance in a normal animal. In seeking an explanation

7 Doerr. In Kolle and Wassermann's Handbuch der pathogenen Mikroorganismen, 1913, II, Part 2, p. 1085.

for the facts it seemed probable that the effective stimulus consisted in the mechanical agitation of the brain To test this view an electric vibrator, designed for therapeutic massage, was used, its flat ebonite disk, rapidly oscillating, being firmly applied for three minutes to the skulls of a series of sensitized guinea-pigs which were slightly etherized The results showed a curious discrepancy

EXPERIMENT 8.—A guinea-pig previously described, which had received a subcutaneous injection of serum July 16, had lost part of its cerebrum August 23, and had recovered from an intravenous injection August 24, was submitted to skull vibrations September 22 The only reaction to the vibration was rubbing of the nose and a slight "steppage" movement September 23 more than 0 5 c c of serum was given intravenously, resulting in dyspnea, peristalsis and cough, but recovery within an hour (This animal received another vibration November 9, followed by intravenous injection November 11, from which it recovered Another intravenous injection was given fourteen days later with recovery, and a fifth injection into a vein was made December 9, without producing symptoms This is a case of either prolonged antianaphylaxis or permanent immunity)

EXPERIMENT 9.—Guinea-pig, male, 820 gm., sensitized July 16 From August 16 to August 31 it received five inhalations of vapor of horse serum without effect September 2 the electric vibrator was applied to its skull for about five minutes There was no reaction except "steppage" movements, nose rubbing and slight incoordination The next day the animal received intravenously 0 75 c c of serum and fully recovered with very slight symptoms (Twenty days later the olfactory lobes were removed The shock was very intense The animal never fully recovered, though it would eat greedily, it died four days after the operation)

The results of subsequent experiments indicate that desensitization caused in the foregoing animals by vibration of the skull was dependent in some way on the treatment they had previously received auxilliary to the primary sensitization Five other guinea-pigs, two of which had been sensitized intravenously, were submitted to vibration and all died in shock when injected the following day by the vein Two of the animals before the toxic injections received no less than six vibration-treatments at intervals of two days The application of the vibrator to the skull is exceedingly drastic treatment and frequently resulted in complete inhibition of the respiration and great slowing and enfeeblement of the heart-beat

The phase of antianaphylaxis produced by the shock of brain operations is very short It passes off in less than forty-eight hours, as was evidenced by three special experiments which it is not necessary to detail That no shock is occasioned by moderate ablation of nervous tissue in the normal pig is shown by the following

EXPERIMENT 10.—Normal male, 360 gm Olfactory lobes and a portion of the anterior cerebrum slightly greater in bulk were removed November 3 There was no reaction whatever (In two days this pig sickened, probably from infection, and died in a week Other normal pigs suffered loss of much greater quantity of brain without permanent disturbance)

In a widely quoted paper, Schurei and Strasmann[8] describe experiments in which they attempt to contravert Besredka's idea that the sole seat of the anaphylactic reaction is in the brain They excised the whole brain, including the basal ganglia. in sensitized guinea-pigs and then gave them serum by intravenous injection The animals died within a period varying from a few minutes to several hours, and the results were supposed to contravert the theory that the brain is involved in anaphylactic phenomena In the light of my observations it is plain that such methods are necessarily incompetent to clear up the subject

DESENSITIZATION OF THE BRAIN THROUGH SECTION OF THE OLFACTORY NERVES

On one occasion, the olfactory lobes were removed from a fully sensitized guinea-pig but the animal manifested no sign of shock whatever In seeking the cause for this departure from the rule that had seemed established, it was found that this pig had, eight days before, been subjected to section of its olfactory nerves It at once became necessary to follow out the suggestion aroused by this development

EXPERIMENT 11.—Six young pigs, weighing about 270 gm each, were sensitized by subcutaneous injection August 29 From four of these, Pigs 1, 2, 3 and 4, the olfactory nerves were cut and wiped out September 16, under very slight etherization There was no reaction except to a very slight degree in one (Pig 2) in which bleeding occurred through the nose due to injury to the cribriform plate September 24, twenty six days after subcutaneous injection, the olfactory lobes were removed from Pigs 1, 2 and 5 (the latter having intact olfactory nerves) Pigs 1 and 2 showed no shock, Pig 5 lay on its side for nearly ten minutes with somewhat convulsive clonic trembling of the legs This shock did not produce antianaphylaxis for the animal succumbed to intravenous injection the next day September 25 the olfactory lobes were excised from Pig 3 There was no shock whatever, the animal sitting up at once when released, though it rubbed its nose and preened the body On the same date there were taken from Pig 4 (olfactory nerves destroyed September 16), both the olfactory lobes and a portion of the anterior cerebrum, about equal in bulk to the combined olfactory lobes The animal lay quietly on its side for two minutes after release from the holder, breathing hard but without convulsive movements, and then sat up with normal appearance except for slight temporary spasmodic nodding of the head No antianaphylaxis was produced, for the animal succumbed to an intravenous injection the next day, September 25 Pig 6, sensitized but with intact olfactory nerves, was made to lose its olfactory lobes and a portion of the anterior cerebrum equal to a single olfactory lobe When released this animal lay on its side for five minutes with clonic convulsions It then sat up and appeared normal for awhile, but soon became restless and the subject of forced movements, which continued until the animal died in convulsions, attended with enormous erections of the penis, after six hours At necropsy the lungs were found collapsed, containing a few hemorrhagic areas but no consolidation

8 Schurei, J, and Strasmann, R Ztschr f Immunitatsforsch, 1912, xii, 143

The experiments show very definitely that in sensitized guinea-pigs the nervous shock following mutilation of the brain is greatly reduced eight or ten days after section of the olfactory nerves It was sought to determine how soon such desensitization of the brain followed on nerve section, and how long it continued

EXPERIMENT 12.—Pig 1, male, 440 gm., sensitized August 29 Olfactory nerves destroyed September 24 Next day the olfactory lobes were removed The animal manifested moderate but decided action, such as relatively slow recovery from the operation followed by nose rubbing and clonic tremblings of the body The shock was not sufficient to produce antianaphylaxis for the pig succumbed in three minutes next day to toxic injection

Pig 2, female, 910 gm far advanced in pregnancy sensitized August 29 Olfactory nerves cut November 5 November 6 the olfactory lobes and a like bulk of cerebrum were removed There was scarcely any shock following the operation, the only sign of anaphylaxis being a slight inspiratory humping of the body setting in ten minutes after release

These results indicate that while the desensitizing effect of olfactory nerve section on the brain may develop within a day, the full influence of the deprivation of olfactory stimuli is not established with certainty till later

EXPERIMENT 13.—Male pig, 500 gm., sensitized August 29 Olfactory nerves cut November 5 November 18 excised olfactory lobes and a similar bulk of cerebrum The animal lay quiet for nearly ten minutes after release and then sat up with no evidence of reaction except for slight rhythmic lifting of the legs Next day the pig succumbed in three minutes to the toxic injection

It is clear from these experiments that the diminished sensibility of the brain following section of the olfactory nerves in sensitized guinea-pigs may be well marked within twenty-four hours and remains in full force for at least thirteen days After longer periods there may always be suspicion of regeneration of the olfactory nerves In the following experiments part of the cerebrum was removed long after ablation of the olfactory lobes

EXPERIMENT 14.—Female pig, 690 gm Olfactory lobes removed August 27 and the space left filled with melted paraffin (in none of several cases in which this was done could the paraffin be detected with certainty after several weeks) Serum was injected subcutaneously August 29 November 3, sixty-eight days after removal of the lobes, under slight ether anesthesia, there was scooped from the anterior cerebrum an amount of tissue estimated as about twice the bulk of the normal olfactory lobes There resulted moderate but distinct shock, with some forced movements, which passed off after about thirty minutes Two days later the animal withstood an intravenous injection of nearly 0.5 c.c. of serum with scarcely any reaction

This experiment indicates that after removal of the olfactory lobes (of course including the attached nerves) the sensitiveness of the brain to laceration has been more or less completely restored in about two months Similar conclusions were derived from other experiments the

conditions of which were more complicated, which it is unnecessary to describe

It might be claimed that the influence on the irritability of the brain apparently exerted by the olfactory sense is not a specific property of this sense but is wielded by any strong sensory impulse or is also a part of the function of other special senses I did not fully grasp, until too late to sensitize new animals, the importance of testing the relation of the visual sense to cerebral irritability. A single experiment, however, went to show that the removal of one eye does not desensitize the brain as in the case after section of the olfactory nerves

EXPERIMENT 15.—Male, 615 gm Sensitized by intravenous injection of 0 5 c c of serum September 6 Under ether anesthesia left eyeball was removed November 6 November 10, under ether, the olfactory lobes and an equal bulk of cerebrum were removed There was moderate but pronounced shock lasting for more than half an hour The shock was not sufficient to protect the pig with anti-anaphylaxis when an intravenous injection was given next day

From the results that have been described in the last two sections of this work it seems impossible to avoid the conclusion that sensitization of the brain and sensitization of the body represent different biologic conditions The former is a labile, the latter a stabile, state The threshold of irritability of the sensitized brain is plainly leveled in response to olfactory sensory stimuli The brain may be completely desensitized without affecting in the least the response of the body to the second, toxic dose of the antigen An attempt to elucidate the teleological significance of these coordinations will be made in the discussion at the end of this paper As is well known, Besredka asserts that the intracerebral injection of 0 25 c c of serum will, in an unanesthetized sensitized guinea-pig, cause speedy anaphylactic death If the conclusions drawn above as to desensitization of the brain following section of the olfactory nerves be correct, it should be possible for an animal so treated to resist Besredka's fatal injection That this is true is proved by the following:

EXPERIMENT 16.—Pig 1, female, 360 gm, sensitized August 29 Olfactory nerves cut and wiped out September 16 September 26, under light ether anesthesia, a trephine hole was made over the cerebrum and there was injected through a hypodermic needle into both hemispheres a total of 0 25 c c of horse-serum The animal was practically out from under the influence of ether during the injection There was but very slight reaction marked by restlessness and some jerking of the head This animal weighed 450 gm November 5, when it received an intravenous injection and died in four minutes

Pig 2, female, 840 gm, sensitized August 29 Olfactory nerves cut November 5 November 8 the skull was trephined over posterior cerebrum and the animal was released until entirely recovered from ether, then, as before, 0 25 c c of serum was injected into the brain There was rather severe shock marked by clonic twitchings of legs, rapid breathing, circular movements weakness of hind legs, gradual recovery after an hour or two This shock was not sufficient

to produce antianaphylaxis, for the animal succumbed next day in the usual way to intravenous injection

Besredka found that intracerebral injections made with very small amounts of serum, or made under deep ether anesthesia, are not lethal, and the following day the animal is found to be antianaphylactic Rosenau and Anderson[9] repeated these experiments on etherized guinea-pigs and found that seven out of eight animals died from the second injection given next day These discrepancies may be accounted for by supposing that the duration of the antianaphylactic state may be less as well as more than twenty-four hours

According to the preceding argument, it should be suspected that ether narcosis affects only the susceptibility of the brain to shock, leaving that of the body unaffected It was found, in fact, that a sensitized guinea-pig etherized to relaxation, died as quickly from intravenous injection of serum as one treated without an anesthetic In the earlier experiments of this work the intravenous injections were all given without an anesthetic for fear of masking the anaphylactic symptoms, but in all the confirmatory tests the animals were subjected to a slight etherization, with little or no distortion of reactive symptoms

Reference was made in the preceding section of this paper to an impression which developed as the result of prolonged experimentation, that the anaphylactic shock caused by the ablation of a definite mass of brain tissue became less intense as the weather turned cooler Such a result might be expected if the olfactory sense, whose excitation probably varies with the external temperature, be indeed, a physiological regulator of brain irritability

In his excellent contribution to the great work edited by Kolle and Wassermann, Doerr summarizes his discussion on the site of anaphylaxis thus "That the real points of attack of the anaphylactic noxa lie in the periphery has been established beyond all doubt"[10]

THE RELATION OF THE OLFACTORY NERVES TO SHOCK IN THE NORMAL BRAIN

The marked dependence of anaphylactic disturbance following operations on the brain of serum-sensitized guinea-pigs on integrity of the olfactory nerves raises the question whether in the normal animal there is not a specific physiological relation between the olfactory sense and irritability of the brain The literature of cerebral physiology makes it obvious that so profound a problem can only be solved by a great

9 Rosenau and Anderson Jour Med Research, 1908, xx, 37

10 "Das die wesentlichen Angriffstellen der anaphylaktischer Noxa in der Peripherie liegen, ist heute wohl uber allen Zweifeln sichergestellt" Doerr in Kolle and Wassermann's Handbuch der pathogenen Mikroorganismen, Ed 2, II, Part 2, p 1078

number of guarded observations Nevertheless, a few experiments were ventured in this field and the results were sufficiently positive to deserve record

Five guinea-pigs were taken which were in very fair condition although they had been inoculated with what was evidently an attenuated strain of tubercle bacilli nearly three months before In three of the animals the olfactory nerves were cut and two or three days later the olfactory lobes were excised together with a quantity of cerebral tissue estimated at three times the bulk of the lobes In making this estimate the olfactory lobes were placed on a sheet of paper and three heaps of equal size, scooped from the anterior cerebrum, were laid near by The operations were performed, as usual, under very light anesthesia As will be seen, the symptoms of shock were much more pronounced in the two animals whose olfactory nerves remained intact up to the time of the operation

EXPERIMENT 17 —Pig, 1, weight 600 gm Excised olfactory nerves November 13 November 15 excised olfactory lobes and about three times their bulk of cerebrum Pig when released lay for ten minutes apparently conscious, but with clonic tremblings of the legs and slight dyspnea Recovered quickly, result, very slight reaction

Pig 2, 485 gm November 13 olfactory nerves cut November 16 the olfactory lobes and part of the cerebrum removed as before The animal lay quietly for six minutes and then sat up without reaction except for some dyspnea, nose-rubbing and rhythmic nodding of the head Result, very slight reaction

Pig 3, 510 gm Olfactory nerves cut November 16 November 18 olfactory lobes and three times their bulk of cerebrum removed Animal tried to sit up in six minutes after release There were no convulsive movements, but the animal ran about the room and sought to hide in a dark corner Normal but dull within an hour Result, slight reaction as shown by apparently forced movements

Pig 4, 510 gm Olfactory nerves intact until time of the main operation November 15, when the olfactory lobes (with nerves) and three times their bulk of cerebral tissue were removed Pig lay for more than ten minutes after release with clonic tremblings It then turned on its back with convulsive doubling of the body It was still unable to sit up in twenty minutes after which there was a convulsive twisting of the body with legs kicking It soon recovered Result, marked but moderate reaction

Pig 5, about 580 gm Olfactory nerves intact until main operation November 16, when the olfactory lobes and cerebral tissue were excised as before Pig lay for ten minuntes after release with clonic tremblings of the legs, then there was a tetanoid twisting of the body Sat up in twenty minutes and manifested apparently forced twisting movements Within forty minutes the only disturbance was shown by rhythmic lifting of the body Result, marked but moderate reaction

In answer to the objection to the use of ether in these experiments it may be said that narcotization was of such light degree that animals in which the olfactory nerves alone were cut would sit up at once without reaction on release from the holder When nerve-cell tissue was excised the animals lay on the side, with or without convulsive twitchings, for

a period apparently proportional to the amount of substance removed, that is, the symptoms were a result of the operation, not of the anesthesia

INFLUENCE OF COCAINIZATION OF THE NOSE ON THE SHOCK FROM INTRACRANIAL OPERATION

The suggestive surgical conceptions of Crile[11] are recalled by these results and an effort was made to modify the shock of brain operations through an attempt to cocainize the olfactory mucous membrane It is well known that a solution of cocain sprayed into the nose may completely abolish the sense of smell temporarily Only a few unconvincing experiments were tried in this direction A 2 per cent solution of cocain was dropped into the nostrils of two sensitized guinea-pigs four times in the course of fifteen hours Then under light ether anesthesia the olfactory lobes and a somewhat greater amount of cerebral tissue were removed from each, with resultant moderate but distinct reaction in each The shock was much less and cleared up sooner than that which occurred in most sensitized pigs subjected to the same operation without cocain Each of these animals succumbed next day to intravenous injection Of four young pigs weighing about 240 gm each, from a sensitized mother, two were treated with several nasal instillations of cocain One of these pigs quickly died with symptoms to be detailed later The other three were subjected to the loss of the olfactory lobes and about three times their bulk of cerebral tissue There was decidedly less prolonged convulsive reaction in the cocainized pig than in the untreated pigs, though the former showed a high degree of nervous excitement From the results of observations already detailed it is obvious that cocain anesthesia should be prolonged for a day or two in order to obtain its desensitizing action, if any, on the brain

CERTAIN ANALOGIES BETWEEN THE EFFECTS OF COCAIN POISONING AND ANAPHYLACTIC SHOCK

Guinea-pigs were killed by nasal instillation of cocain and by subcutaneous and intravenous injection Five unsensitized pigs were given intravenous injections of cocain in about 4 per cent solution Whether the poison was injected slowly or rapidly the result was the same, a speedy diminution in the amplitude of respiratory movement, and death without convulsion The heart continued beating for many minutes after respiratory arrest just as it does in anaphylactic death The lungs were found collapsed at necropsy

11 Crile, G W Boston Med and Surg Jour, 1910, clxiii, 893

Three young pigs, offspring of a sensitized mother, weighing 180 to 240 gm each, were killed by the repeated instillation in the nose of a cocain solution of from 2 to 4 per cent The symptoms of poisoning came on very suddenly The animal would fall on its side, the head was tonically retracted, frequent and well-marked opisthotonus and clonic convulsions of legs and body occurred The same series of events occurred when subcutaneous injections of cocain were given This was done in the case of seven animals, four of which were serum-sensitized, but the element of respiratory disturbance was more marked than when the poison was given by the nostrils The retraction of the head was a characteristic symptom, the chest would be distended and the respiratory movements very rapid and so shallow as to be hardly visible An animal might recover after remaining more than half an hour apparently *in extremis* The convulsive phenomena witnessed in these animals were exceedingly suggestive of those seen in severe anaphylactic shock But the particular object of these experiments was to follow out an observation that had been made on the pig from a sensitized mother, which had first succumbed to cocain by the nose The lungs of this animal were found, post mortem, to be markedly distended, almost as much so as after anaphylactic shock In two other cases this extraordinary post-mortem distention of the lungs was found, although usually the lungs were well retracted from the heart on the ventral side but maintaining apparently a condition of semi-expansion dorsally In two normal guinea-pigs killed by cocain subcutaneously the post-mortem lung-distention was less striking than in the sensitized or offspring of sensitized pigs That the post-mortem distention of the lungs did not depend on the terminal convulsions seemed evidenced by the state of two sensitized pigs which were injected with strychnin; in these the lungs were found completely collapsed at necropsy The expanded lung after death from cocain offers marked differences from the expanded lung after anaphylactic death The former is pale, the latter congested Both remain distended when excised, but the former crepitates little and collapses but slightly when squeezed in the fingers, the latter does both In that minority of cases in which the lung remains distended after death by cocain, its behavior suggests a rubber-like structure The phenomena described in this section recall the similar, though still more striking, effects described by Dale and Laidlaw[12] as following the injection into guinea-pigs of betaimidazolylethylamin They call attention to the anaphylactic-like changes produced by this substance

12 Dale and Laidlaw Jour Physiol , 1911, xliii, 182

REACTIONS OF GUINEA-PIGS TO HORSE-SERUM ADMINISTERED THROUGH THE NOSE AND BY INHALATION IN SPRAYS LOCAL AND GENERAL ANAPHYLAXIS

Comparatively few investigations seem to have been made with a view to determining the effect of the application of antigens to the unaltered peripheral epithelium The reaction to the tuberculin test of von Pirquet involves wounding of the outer skin Administration of antigens by feeding, especially by means of the stomach-tube, greatly complicates the experimental conditions In an important paper, Dserzgowsky[13] describes experiments in which plugs of cotton wool moistened with the toxin of diphtheria were applied to the mucous membrane of the nose and the toxin itself to the general respiratory tract through the inhalation of sprays Experiments were performed on horses and on the author himself It was asserted that in these ways active immunity, both local and general, could be harmlessly induced Blumenau,[14] following this suggestion, inserted for thirty minutes tampons moistened with diphtheria toxin, diluted to one-third strength, into the nostrils of children After twenty such treatments diphtheria antitoxin was found in the blood Practically the same result was obtained by Scheweleff[15] in experiments on dogs A C Klebs,[16] in his history of small-pox inoculation, mentions an ancient custom among the Chinese of protecting against small-pox by putting its virus into the nose

Busson[17] describes experiments in which guinea-pigs sensitized to horse-serum were inclosed in a tight box and made to inhale a finely divided spray of horse-serum, ten times diluted, for a period of two hours or more As far as the description can be understood there were very slight but positive signs of anaphylactic reaction The rise of temperature within the box and the deterioration of the quality of the air must have introduced important complications into the experiment My results confirm, in general, those of this author

Schlecht[18] records a series of researches which show that injection of a foreign serum into the body causes in the blood a great temporary increase of eosinophils, and in guinea-pigs of mast-cells as well, due to increased activity of the bone-marrow The administration of spray

13 Dserzgowsky, S K Ztschr f Immunitatsforsch (abst), 1910, iii, 602
14 Blumenau, N R Ibid , 1910, iv, 353
15 Scheweleff, P P Ibid , 1910, v, 1085 (Ref Bd)
16 Klebs, A C Bull Johns Hopkins Hosp , 1913, xxiv, 69
17 Busson, B Wien klin Wchnschr , 1911, xxiv, 1492
18 Schlecht, H Kong f inn Med , 1912, xxiv, 416, Deutsch Arch f klin Med , 1910, xc, 308, ibid , 1912, cviii, 405, Arch f exper Path u Pharmakol , 1912, lxvii, 137

inhalations of horse-serum to guinea-pigs, either sensitized or normal, was followed by a high grade of eosinophilia localized in the lungs

In the discussion of Schlect's paper, read before the Congress for Internal Medicine, Schittenhelm said that he had not been able to repeat the results of Friedberger, who had produced aseptic pneumonia in sensitized guinea-pigs by inhalations of serum

Ishioka[19] made injections of serum through a fine cannula directly into the trachea of sensitized guinea-pigs Out of forty animals two died at once in anaphylactic shock, the others showed symptoms but recovered. In a few cases there was produced interstitial pneumonia with much emphysema In most cases there resulted true lobar pneumonia indicated by patches of infiltration which could even involve a whole lobe

My method of bringing the serum in contact with the respiratory epithelium differed radically from that described elsewhere Some two dozen guinea-pigs were treated in two groups, in one the treatment consisted in instilling fresh horse-serum into the nose by dropping it from a hypodermic needle, in the other the serum was breathed as a fine spray In the first case the animal was gently held by the hand on its back, from an all-glass hypodermic syringe with easily moving piston and a coarse, blunt needle, drioplets of serum to the amount of from 0 25 to 0 33 c c were allowed to fall into one or the other of the nostrils As a rule, only one side of the nose was utilized at each sitting Various animals were treated daily or at intervals of one, two or three days

The serum spray was produced in a commercial "vaporizer" actuated from a compressed-air tank The spray thus formed was exceedingly fine and resembled the vapor from a gently boiling teakettle A funnel made of stiff paper was applied over the nose and mouth of the guinea-pig as it sat on a table The nozzle of the spray apparatus was closely applied within the smaller but open end of the funnel The spray was applied at intervals similar to those of the instillations and the sittings lasted, as a rule, about three minutes The serum was often used in full strength, in dilutions of one-half or one-quarter strength the results appeared the same Theoretical objections against this method of procedure seem invalid in the light of the reactions obtained

Each group of guinea-pigs was composed of four subdivisions (1) normal animals, (2) animals sensitized by the subcutaneous injection of serum, (3) unsensitized animals which had weeks before been relieved of their olfactory lobes, (4) animals which, after the removal of their olfactory lobes, had been subcutaneously sensitized

19 Ishioka, S Deutsch Arch f klin Med, 1912, cvii, 500

SPECIAL REACTIONS TO THE NASAL INSTILLATION OF SERUM

When horse-serum is slowly dropped as described into a nostril of a guinea-pig for the first time, there is usually no disturbance whatever The fluid is quickly sucked into the nose, and not until a considerable quantity has been introduced is there any expulsion, and this is evidently mechanical Late in the sitting, taste and probably swallowing motions are manifested The nasal canal in a medium-sized guinea-pig has a length of nearly 5 cm and therefore it is not likely that the first droplets reach the pharynx Even with the second treatment, after two days, signs of active resistance to the instillation of serum may appear, brisk expiratory sniffing movements more or less resist the entrance of serum or expel it from the nostril

These minor phenomena are physiologically significant but are difficult to interpret because they may occur with the instillation of normal saline or solutions of cocain at the very first administration It was noted, however, that active resistance to the instillation of serum appeared uniformly earlier in animals with intact olfactories than in those deprived of their olfactory lobes In animals treated every alternate day, my notes record signs of great nasal irritation in normal pigs at the second instillation, while in specimens without olfactory lobes it was not until the fourth treatment, after six days, that active reactions were produced

Previous subcutaneous sensitization had no perceptible influence in accelerating the reactions One definite and undoubtedly reflex response to the instillation was regularly obtained from certain animals This was an excess of salivary secretion While the mouths of practically all pigs which showed signs of irritation against the serum became unusually moist, in a certain number saliva would well up between and drool from the lips This reaction held constant in the same pigs through a prolonged series of observations The reaction was usually positive in normal pigs It was obvious, however, that saliva accumulating in the mouth was frequently removed by swallowing and thus failed to be noted The reaction can hardly be regarded as other than a sign of specific sensitization It occurred both in animals previously subcutaneously sensitized and in unsensitized animals, whether with or without olfactory lobes It did not occur through the instillation of normal salt solution even when it was constantly produced by serum A single nasal instillation with serum evidently induces sensitization Thus, October 11, two normal guinea-pigs each received 0 25 c c of serum in both nostrils The only effect witnessed was the excitement of taste movements

Sixteen days later, October 27, the treatment was repeated There was now considerable nasal irritation as shown by sniffing and active

expulsion of serum from the nose and rubbing of the nose with the paws, and in each case saliva welled from the mouth Two days later. October 29, one of these animals received an intravenous injection of serum, there was *no reaction.* Five other nasal instillations were given to each pig from November 12 to November 20 The resulting symptoms were the same as those elicited October 27, except that now the animal which had not received the intravenous injection ceased to respond to the instillation by the secretion of saliva Finally, November 23, both animals received intravenous injections and both died in typical shock This account anticipates my discussion, but clearly indicates that *local sensitization of the nasal mucous membrane may occur independently of and precede general sensitization*

The symptoms thus far described as resulting from nasal instillation of serum are minor reactions which it is possible to confuse with those following non-specific stimuli But there were extraordinary results obtained in the case of a single animal, which, though obviously somewhat dependent on idiosyncrasy, are detailed because they differ only quantitatively from reactions characteristic of this group of experiments A guinea-pig weighing 435 gm was treated by the nostrils every second day During the tenth treatment, eighteen days after the first, the animal suddenly choked, loud rattling râles with occasional cough accompanied the deep and labored respiration The phenomena strongly suggested those of the severest form of bronchial asthma in man It was several hours before the pig recovered sufficiently to eat It had been noticed that spasmodic partial closure of the nostril had resisted the intake of serum Subsequent administrations of serum each second day provoked similar though milder symptoms, an excessive salivary secretion becoming a relatively more prominent reaction The symptoms could not have been due to the flow of serum directly into the trachea, for they appeared while still too small a quantity of serum had been applied Later results with other animals leave no doubt as to the anaphylactic character of the disturbance

THE EFFECT OF THE INHALATION OF SERUM SPRAYS

The statement was made in the beginning that the initial exhibition of vaporized serum, according to the method described, provoked no symptoms whatever Guinea-pigs previously sensitized by subcutaneous injection reacted apparently as negatively to the inhalation as did normal animals

A remarkable difference, however, presented itself in the behavior of those guinea-pigs which received the serum inhalation after a course of serum instillations by the nostril In these cases there was an immediate response to the first inhalation in the form of a violent

dyspnea, with occasional cough, asthmatic in its urgency but characterized by rapid respiratory movement The attack would develop suddenly within about a minute of beginning inhalation and would not subside for from thirty minutes to several hours Unfortunately no attempt was made to determine whether a phase of antianaphylaxis succeeded recovery from the attack of dyspnea Repetitions of inhalation at intervals of two days or more invariably provoked the same response

EXPERIMENT 18.—Two young pigs were treated by instillation of serum in the nose every third and every fourth day from May 28 to July 12 Serum inhalations were given to one July 17, and to the other August 7 Both animals exhibited the reaction described, namely, violent, rapid dyspnea, interrupted with occasional deep breaths and cough

These, together with subsequent observations, make it clear that local sensitization of the nasal mucous membrane to horse-serum may be induced without the excitement of any very marked reaction An animal thus peripherally sensitized responds immediately to serum inhalation with characteristic dyspnea, while one sensitized subcutaneously does not My friend Dr Hillkowitz kindly tested the blood drawn from the heart of one of the animals described, for precipitins to horse-serum The dilutions used were 1 1,000 and 1 10,000 The results were negative, as might have been expected from the similar observations of Rosenau and Anderson[20] An excellent opportunity seemed presented in these animals to determine whether the olfactory mechanism played any rôle in the serum reactions

EXPERIMENT 19.—A female pig, 645 gm, which had regularly responded to the inhalation, had its olfactory nerves divided August 25 Four days later, and during subsequent exposures, the serum inhalations produced symptoms identical with those produced when the olfactory nerves were intact, though possibly the latent period of reaction was longer September 2 the olfactory lobes were excised in this pig without special reaction (because of previous section of the nerves) Two days later a serum inhalation provoked the characteristic dyspnea which began after the vaporization of thirty or forty drops of serum September 10 this animal succumbed, in less than five minutes, to the intravenous injection of 0 5 c c of serum

It was clear in this case, and abundantly confirmed later, that the peripheral application of serum not only sensitized in a peculiar way the peripheral respiratory epithelium but produced general sensitization as well These results were to have been anticipated from the findings of other observers who used different methods General body sensitization develops gradually during a course of local treatment with serum and apparently is preceded by the acquisition of local anaphylaxis An effort was made to trace the development of the reactions of local and general anaphylaxis

20 Rosenau and Anderson Bull Hyg Lab, U S P H S, 1906, No 29, p 35.

EXPERIMENT 20.—Two normal male pigs, weighing 360 gm. each, October 11 were made to inhale, with intermissions, for about ten minutes the vapor of a 50 per cent. dilution of serum. There was slight temporary dyspnea, undoubtedly mechanical in origin and due to the long exposure to the vapor (the usual treatment occupied three minutes or less and the specific reaction commonly occurred within a minute or two). October 26, fifteen days later, the pigs having increased in weight, a similar less prolonged treatment was given. There was no specific reaction, although Pig 1 breathed hard for a while ten minutes later. October 29, three days after the second and eighteen after the first inhalation, Pig 2 received, without ether, 0.5 c.c. of serum intravenously. There was no reaction. November 12 both pigs were again sprayed with serum of 50 per cent. dilution. Almost at once Pig 1, which had not received the intravenous injection, was seized with violent, rapid panting. This passed off in about fifteen minutes. Three days later in the morning this pig was found dead. I cannot account for the fatality except as a result of the treatment combined possibly with low resistance to the cool air of the night. Necropsy showed the olfactory lobes apparently much softened. The lungs were collapsed and congested but without hemorrhagic areas. Pig 2, which had received intravenous injection October 29, received four serum-inhalations from November 15 to November 20. No dyspnea was caused by either treatment. This animal was given injection of serum by the vein November 23, and died with violent convulsive and bucking seizures.

These observations show that a single inhalation of serum by a normal guinea-pig provokes, when repeated fifteen days later, hardly any perceptible anaphylactic response. A third spraying, performed seventeen days after the last, produces a well-marked reaction. Intravenous injection of serum during these observations may have prevented, it certainly did not exaggerate, the reactions. It was found, as might have been expected, that guinea-pigs treated in precisely the same manner showed more or less individual differences in behavior. In contrast with the results of the last series of experiments, the following observation shows that possibly response to the local application of the antigen may be intensified by previous general sensitization.

EXPERIMENT 21.—Male, weight 570 gm. Olfactory lobes removed August 26 and the space left filled with melted paraffin. Sensitized subcutaneously with 0.33 c.c. of serum August 29. Received a prolonged inhalation of serum spray September 25 followed by no specific reaction. Inhalation was given again October 10, fifteen days later. A violent rapid dyspnea, which had not disappeared after an hour, immediately resulted. The treatment was repeated three times on alternate days, with identical response except that the latent period of reaction increased to a minute or two.

This experiment demonstrates again that response to the inhalation does not demand an intact olfactory apparatus.

I will now review rapidly the history of eighteen guinea-pigs, including individuals which were subcutaneously sensitized or normal and with olfactory lobes intact or removed. Half were treated primarily with instillations of serum into the nose and half with serum inhalations. Each group was again subdivided into two parts, the animals of one subgroup had serum dropped into the nose for four times on alternate days and those of the other for four times every fourth day.

The result was more or less active reaction with minor symptoms such as have already been described After a resting interval of twelve days by the first group and of six days by the second group, which followed the last treatment in each, all of the nine "instillation" pigs were again given serum by the nose To the local symptoms of nasal irritation and salivary secretion in certain animals was now added a more general reaction marked by more or less obvious dyspnea together with rattling, apparently bronchial râles Of the two pigs in which the latter symptoms appeared, both had been subcutaneously sensitized and one had lost its olfactory lobes Both râles and dyspnea were manifested only by animals which had received serum by the nose every fourth day It is worth noting that when the group of animals treated in four sittings by serum dropped into the nose were given a like treatment eighteen days after the first dosage, the anaphylactic response was much more marked in animals which had received the preliminary instillations every *fourth* day

In a parallel series of observations carried out with spray-inhalations, the reverse was noted, the strongest reactions occurred in animals receiving the four initial inhalations on *alternate* days Five of the "instillation pigs" were given inhalations of serum on the day following the treatment which had produced so marked a reaction The result was the production of a marked specific dyspnea in two of these animals It is worth noting that of two pigs which had received no serum subcutaneously and from which the olfactory lobes had been removed, one responded by dyspnea to the spray and one did not Of the two pigs not operated on, likewise without subcutaneous serum, neither responded This experiment shows again that local sensitization of one part of the respiratory tract involves sensitization of the rest, though, as will be pointed out presently, there are marked local signs which distinguish the dyspnea produced by inhalation of serum in spray from that caused by its instillation in the nose

Rapid dyspnea was the one sign of anaphylaxis observed in the inhalation experiments It could be produced in nearly all animals, but some individuals responded much more readily than others One animal which was made dyspneic by the inhalation, failed to react when sprayed daily, until the eighth day, when it responded again In general, the daily administration of inhalations produced no response, though one pig which had received its first inhalation about two weeks previously responded each time to inhalation given every forty-eight hours Of four animals which had received four sprayings on alternate days, from October 10 to 16, only one had shown marked reaction They received another inhalation October 26 and each responded strongly — in fact the dyspnea in two cases was of desperate urgency

Of these animals only two had been sensitized with serum subcutaneously One of them had been deprived of olfactory lobes

Five pigs which had received four inhalations from October 10 to 22 were likewise treated with the spray, October 26, but all responded very feebly These animals were again given inhalations, November 2, and the dyspneic response was decidedly more vigorous though still much less marked than in the group treated after a longer passive interval These results suggest, of course, the similar findings when anaphylaxis is studied through subcutaneous injections given at close intervals

In a previous experiment it was shown that guinea-pigs sensitized by serum dropped into the nose could be made dyspneic at once by subjecting them to a spray of serum The converse of this experiment was now performed Four pigs that had reacted to the spray by dyspnea were given serum by the nose In one there was reaction with only minor symptoms, but in the other three after long latent periods, five to ten minutes, rattling râles developed, which seemed to emanate from both the bronchi and the nose, there was also more or less cough

As already mentioned, there is a marked difference in the quality of the anaphylactic response to the serum spray and serum instillation In animals subjected to the former there are no râles, however violent the dyspnea In animals responding strongly to the instillation, râles, which may be very loud and lasting, may occur with a variable element of dyspnea The difference depends, probably, on the volume of secretion excited, that is, when a pig sensitized by instillation responds to the spray it is with a dry dyspnea, when a pig sensitized by the spray responds to instillation of serum droplets it is with a moist dyspnea In later observations it was found that, in the shock resulting from the toxic intravenous injection, the influence of the "local sign" of the site of sensitization still held good A pig which had been sensitized by the spray breathed very fast in its anaphylactic throes but developed no râles Others which had been sensitized by instillation developed râles with, usually, less rapid breathing under the same conditions

All the animals employed in these experiments were finally submitted to the supreme test of intravenous injection of serum By this means it was sought to determine to what degree sensitization of the body could be developed by the peripheral application of serum, if at all, and also whether such application could essentially modify sensitization already induced by serum injected under the skin The intravenous injections were given from twenty-nine to thirty days after beginning of the local treatment and after general sensitization had certainly been secured Of the twenty and more animals available, four survived the first intravenous injection of serum

Of eleven pigs treated by nasal instillation of serum three survived the first "toxic" injection One of these belonged to a group of three from which the olfactory nerves and lobes had been removed but which had received no serum subcutaneously This animal had been given serum by the nose four times at intervals of four days, from October 9 to October 21, and again on October 27 The creature had manifested plain signs of sensitization Treated with serum spray, October 28, it became violently dyspneic It was given 0 25 c c of serum intravenously November 10 The animal was badly shocked in a manner peculiarly different from the ordinary anaphylactic seizures, that is, there were no clonic convulsions but the respiration was exceedingly rapid and made audible by râles After about an hour, when the animal had apparently recovered, it suddenly suffered a fleeting twisting convulsion of the body Thirteen days later, November 23, this animal received a second intravenous injection of more than 0 5 c c of serum Great shock resulted with, at the outset, no sign of convulsion except clonic twistings The respiration was very rapid and bronchial and nasal râles were loud Finally, after sixteen days, December 9, a third intravenous injection of about 0 75 c c of serum was given There was no anaphylactic reaction but great muscular weakness Slight clonic twitchings set in after several hours, the weakness increased and the animal died in about twenty hours Animals of this class also differed from those in ordinary anaphylactic reaction in that they appeared to be conscious even while lying on the side in deepest shock

Of the other two "instillation pigs" that survived intravenous injection, both belonged to a group of three normal animals which had neither been operated on nor received serum subcutaneously Their local treatment was similar to that of the last animal described except that neither was sprayed Both of these animals, especially the second, had manifested slight but apparently unmistakable anaphylactic reaction to serum dropped into the nose The first of the two was given 0 33 c c of serum intravenously November 8 The creature remained phenomenally quiet throughout the injection There was no reaction whatever The injection was repeated with 0 5 c c of serum, November 24 result, violent convulsions and typical death I am inclined to interpret this case as further evidence that local sensitization may be achieved without producing any demonstrable general sensitization The second surviving pig of this lot had especially reacted to instillation of serum by abundant secretions of saliva, an apparent sign of sensitization Given an intravenous injection of 0 5 c c of serum, November 23, there was no reaction whatever (the animal was a female far advanced in pregnancy) Sixteen days later, the animal having borne a litter of young a week previously, a second intravenous injection of nearly 0 5 c c of serum

was given There was no reaction except for rapid, jerky breathing and a few râles Special stress deserves to be laid on the fact that this animal had achieved its wonderful power of self-protection by a course of treatment which had provoked exceedingly slight signs of anaphylaxis

The single "inhalation-pig" that survived an intravenous injection of serum belonged likewise to a group of three which had neither been subcutaneously sensitized nor operated on This animal did not react to repeated inhalation treatments until several had been given, then the response was slight but positive as to anaphylaxis November 8, 0 5 c c of serum was given intravenously The reaction was moderate but distinct At the outset respiration was deepened and there were slight convulsive movements Then the breathing became very rapid, interrupted by occasional very deep breaths, and so continued for half an hour There were no râles as in the instillation pigs November 24, sixteen days later, the treatment was repeated Deep, rapid respiration with strangling cough came on at once The animal died in five minutes *without having exhibited respiratory râles or any convulsions*

It will be noted that none of the animals which had been previously sensitized by the subcutaneous administration of serum survived the toxic injection The conclusion is that general sensitization so produced is not essentially modified by the efficient antigen applied to the peripheral epithelium, although general sensitization is undoubtedly achieved in this way That this non-interference of the serum, peripherally applied, with the previous general sensitization, is not dependent on the time intervals of its application, seems shown by the fact that six "instillation" and four "spray" pigs received a course of seven daily treatments extending to within three days of the toxic injection, but only one survived it In other cases the interval between the last local treatment and the toxic injection extended to more than twelve days It is clear also that the olfactory apparatus, as far as represented by the olfactory lobes and intracranial nerves, is not essential to the systemic effects of serum locally applied In fact, the normal animals apparently resisted general sensitization in an active way except as to the secretion of saliva Of a group of animals reacting in about the same way to local treatment, one might survive and the others succumb to the toxic injection

There was nothing in the results obtained by me to suggest any change in the respiratory epithelium resembling the "phenomenon of Arthus" (increasingly morbid local changes following repeated subcutaneous injections), which has been carefully investigated by Nicolle [21] This subject was not, however, definitely investigated

21 Nicolle, M Ann de l'Inst Pasteur, 1907, xxi, 128

The statements of Friedberger, Ishioka and Busson in regard to pneumonic changes in the lungs of sprayed animals were, to a degree, confirmed Such lungs at necropsy frequently revealed air-free hemorrhagic areas of limited extent The affected portions when removed would sink in water, but they were soft and not resistant as in pneumonic consolidation The post mortem change was by no means constant among sprayed pigs and was also found in animals dying from toxic injections, which had not been sprayed at all

POSSIBLE ATROPHY OF THE OLFACTORY EPITHELIUM

Because of its importance I venture mention of a theme on which investigation was started but abandoned early in this work In animals deprived of their olfactory lobes and even in some under other and functional conditions, there seemed to be a well-marked atrophy of the olfactory mucous membrane as disclosed by cutting away the roof of the nasal cavities The subject was pursued no further than to give a basis for special research

THE EFFECT ON TUBERCULOUS GUINEA-PIGS OF TUBERCULIN APPLIED TO THE PERIPHERAL EPITHELIUM

It was, of course, a surface thought that some useful therapeutic purpose might be served by treating a systemic affection through the peripheral epithelium Tuberculous guinea-pigs were the subjects and the old tuberculin of Koch was the remedy Six young guinea-pigs were inoculated with what proved to be a very attenuated culture of tubercle bacilli, kindly furnished me by Dr G B Webb of Colorado Springs, July 25 Three of the animals were inoculated in the peritoneum and three beneath the skin Two were used as controls and four were treated The treatment consisted in dropping into the mouth of two subjects, in such a way as to excite the maximum taste-sensations, or in instilling into the nose (two subjects) dilutions of old tuberculin usually of from 0 5 to 1 per cent Treatment given was from every day to every fourth day Treatments were begun more than six weeks before the inoculations and were continued for more than two months afterward Superficial observation could detect no particular reaction to the treatments except the excitement of slight dyspnea by spraying (which was practiced later) One of the treated pigs had to be killed, October 23, because of a tuberculous swelling occluding the urethra The other pigs all gained in weight and were sacrificed for specific purposes nearly four months after their inoculation with tubercle bacilli Post-mortem examination left no manner of doubt as to the effect of treatment The extent and intensity of the tuberculous processes were much greater in the treated animals than in the controls

DISCUSSION AND SUMMARY

Certain facts recorded in this investigation seem to furnish evidence which must be considered in further discussions of the old problem of the physiological requirements in ventilation The trend of scientific opinion has lately been to ascribe the deleterious properties of rebreathed air solely to the untoward physical conditions of heat and humidity usually involved Rosenau and Amoss[22] recently published experiments tending back to the old "chemical view' of the noxiousness of rebreathed air Their work has been controverted in literature not available to me

The observations, detailed above, on the desensitization of guinea-pigs when exposed to the smell of horses, seem to establish beyond question, if such proof were needed, that animal effluvia when inhaled can produce at once profound systemic disturbance It is usually taken for granted that "ventilation" has the same biological import for all people On the contrary, it may well be that people suffering from various ailments may find in rebreathed air noxal elements specifically related to their infections (as did my guinea-pigs in the stable) Such a conception, of course, vastly complicates the biological consideration of ventilation For many years I have noticed in carrying out physical examinations of the sick that the subjects of pulmonary tuberculosis appeared especially prone to avoid, as far as possible, the inhalation of the examiner's breath, it has seemed to me an instinctive effort toward specific self-protection

Evidence has been brought forward which indicates a peculiar immunological relation between the central nervous system, or at least the brain, and the body The brain can be sensitized and desensitized an indefinite number of times without essentially changing the susceptibility of the body (Besredka records three desensitizations in one animal within a few days) This desensitization of the brain after horse-serum injections may be brought about by a number of different agencies,[22] the specific antigen, as used by Besredka, or something allied to it and contained in the effluvia of horses, or, as I have shown, by cutting the olfactory nerves, lacerating the brain tissue or even by setting up mechanical vibrations in it Rapid desensitization of the brain is marked by a more or less profound nervous shock followed by a brief period of antianaphylaxis during which the animal is absolutely protected from the poisonous antigen The nerve impulses producing this remarkable somatic change must operate on the living tissues It appears probable that these nerve impulses cause the tissues, as it were, to open their receptors for the specific antibody in the blood

22 Rosenau and Amoss Jour Med Research, 1911, xxv, 35

23 Kolle and Wassermann Handbuch der pathogenen Mikroorganismen, II, Part 2, p 1086

If this "allergin" is removed from the circulation an animal has become essentially normal as regards reactivity to that antigen Fixed in the tissues the allergin combines with more or less antigen contained therein and we witness a degree of shock proportional thereto It is significant that all observers seem to agree that the allergin diminishes greatly or disappears from the circulation during the phase of anti-anaphylaxis [24] According to the conception presented here the allergin is released from the tissues after a longer or shorter interval and, when returned to the circulation, susceptibility to the antigen is restored

The widely accepted view of Friedberger[25] that antianaphylaxis depends on the saturation of the anaphylactic antibody with the newly introduced antigen cannot explain the condition when caused by brain mutilation, nor does this author's conception of the two states of non-specific and specific antianaphylaxis clear the matter In fact, Bessau[26] makes a strong stand against Friedberger's theory and concludes that antianaphylaxis results from a reaction of the organism through which its irritability is depressed as regards the anaphylactic poison In the masterly and lucid monograph of the Vaughans[27] it is held that the important factor which determines the refractory state of the reinjected animal "is the changed relation between the amount of ferment and the substrate," that is, the small amount of antibody remaining in the blood of the antianaphylactic animal becomes functionally inoperative in the presence of a great excess of antigen This view, also, fails to account for the facts I have detailed Evidence has been developed to show that the irritability of the brain, using this term in a broad physiological sense, is to an extraordinary extent dependent on olfactory stimuli The teleological interpretation of these coordinations is irresistible An animal smells many agents to which his body is sensitized and which, if eaten, would act as poisons *per se*, but through the olfactory sense the brain is sufficiently stimulated by the odor of the antigen to discharge impulses which set up in the body a state of antianaphylaxis during which the intrinsically poisonous substance may be ingested with impunity The reaction of my guinea-pigs in the stable gives substantial support to such a conception The possible relations of these conceptions to human physiology, psychology and surgery are obvious The experiments with local applications of serum to the nose or by inhalation of a spray show clearly that sensitization of the respiratory epithelium develops after a definite latent period whose length does

24 See the valuable paper by Anderson and Frost, Bull Hyg Lab, U S P H S, 1910, No 64

25 Friedberger Ztschr f Immunitatsforsch, 1909, ii, 208

26 Bessau, G Centralbl f Bakteriol, 1911, lx, 637

27 Vaughan, V C, Vaughan, V C, Jr, and Vaughan, J W Protein Split Products in Relation to Immunity and Disease, 1913, p 312

not appear to be influenced by previous general sensitization of the body

In contrast with this relative independence of central and peripheral sensitizations it is found that sensitization of the nasal respiratory tract involves the pulmonary epithelium, and *vice versa,* though each reacts with its own "local sign" The local reactions of the animal inevitably suggest that local anaphylaxis is a state of active resistance against absorption of the antigen Transferring this idea to bacterial focal infections in the interior of the body, we get a more reasonable explanation of the beneficial results which often follow the injection of antigenous bacterial bodies, these can come into intimate contact with tissues capable of antibody formation from which the bacteria of the infective foci are debarred Finally, the proof is once more absolute that serum dropped into the nose or given by inhalation sensitizes the body irrespective of the olfactory apparatus The obvious explanation of the fact is that the antigen is absorbed by the circulation, as is supposed to be the case when serum is introduced into the rectum [28] But "obvious explanations" are especially dangerous Unfortunately, I have not been able to show that such peripheral application of the antigen in any way influences the reactions of a subcutaneously sensitized animal to the toxic intravenous injection This seems a surprising fact in view of the accepted teaching that repeated injections of sublethal doses of serum produce in an animal a resistance, if not full immunity That some peculiar status is occupied by the phenomena induced by application of serum to the respiratory epithelium seems shown by the fact that in two cases unsensitized guinea-pigs, from one of which the olfactory lobes had been removed, were somatically sensitized by this means and that they thereafter withstood several toxic injections separated by intervals of about two weeks Experimental analogies have been presented for the anaphylactic phenomena of hay-fever and bronchial asthma It is shown that both a general and a local sensitization are to be considered, interdependent, but each having an autonomy

CONCLUSIONS

Briefly summarized, the following conclusions may be drawn

1 Guinea-pigs sensitized to horse-serum may be desensitized by residence in a horse-stable It is probable that this reaction may be rapidly achieved through the olfactory sense or more slowly by absorption through the respiratory mucous membrane The period of anti-anaphylaxis is of less than two days' duration The converse of this,

28 Vaughan, V C Vaughan, V C, Jr, and Vaughan, J W *Protein Split Products*, 1913, p 259

sensitization of normal guinea-pigs in stable air, I have not been able to achieve

2 Removal of part of the brain of a sensitized guinea-pig produces much more severe shock than the same amount of injury in a normal animal This shock is apt to be followed by a period of antianaphylaxis of short duration

3 Section of the olfactory nerves within the cranial cavity preceding the brain operation by a day or more abolishes the tendency to shock Such nerve section in the normal animal also seems to reduce the shock arising from definite brain injury

4 Depression of the olfactory sense may be produced through cocain and the symptoms of poisoning with this drug resemble those of anaphylaxis

5 The body as a whole may be sensitized through the peripheral application of horse-serum after a definite latent period The time required to sensitize the peripheral epithelium has not been found to be materially altered by previous general sensitization A normal animal sensitized by local treatment may resist large intravenous injections of serum repeated at intervals of two weeks

6 Sensitization of one area of the respiratory epithelium involves sensitization of the rest, though the local signs of expression are different

7 The evidence indicates that local anaphylaxis, induced through the periphery, precedes the development of general sensitization

8 Tuberculous guinea-pigs were made worse when treated by instillations or sprays of old tuberculin

Besides the expression already made regarding Dr Powell, it is a pleasure to acknowledge my indebtedness to my colleagues, Dr W C Mitchell and Dr P Hillkowitz, for many favors, and to Dr Auer of the Rockefeller Institute for hints as to technic

1360 Vine Street

METABOLISM IN DEMENTIA PRAECOX*

ELLISON L ROSS, PH D
HOSPITAL, ILL

The findings reported in this paper are the result of work suggested by Dr H D Singer, director of the institute, and begun by A F Wussow in this laboratory Pighini[1] and Statuti[2] claimed from their data that the metabolism of dementia praecox was not normal Wussow[3] and Barnes[4] did not obtain results that confirmed the findings of Pighini and Statuti Folin[5] found no evidence of an abnormal metabolism in dementia praecox

TESTS

The plan of the tests was to determine the income and the outgo of nitrogen, sulphur, phosphorus, calcium and magnesium in cases of dementia praecox Tests were made at various times of the year and various diets were used (Table 1)

The method of preparing and preserving the food used in these tests made it possible to have but one sample of food for analysis and to give the patient a constant diet For example, when hamburger was used as a part of the diet, sufficient very finely ground hamburger was cooked and then again very finely ground and mixed Weighed amounts sufficient for a meal were put in ice-cream boxes and stored in a room which was maintained below 0 C (32 F) In the preparation of a meal one package of hamburger was put into a bowl with a small amount of water, set in a steam oven and warmed Coffee, when used, was prepared by making up a liter or more of a very concentrated solution of commercial soluble coffee Ten cubic centimeters of this were sufficient to make two cups of coffee This amount was put into a pitcher with 20 c c of condensed milk and when the meal was ready to take to the ward, a couple of cups of boiling distilled water were added to each pitcher

There were many difficulties to be overcome in doing metabolism work in cases of dementia praecox One of the difficulties was with the feces Cases of chronic or temporary constipation were frequently met

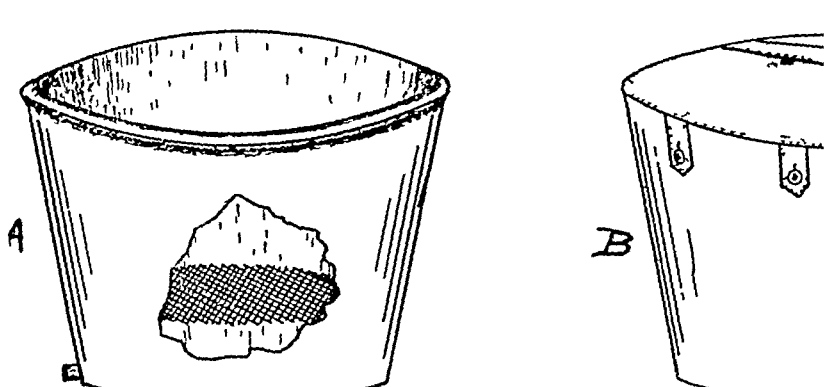
* From the Illinois State Psychopathic Institute
* Submitted for publication Feb 16, 1914

1 Pighini Arch Neurol and Psychiat, 1909, iv, 220
2 Statuti Am Jour Insanity, 1910, lxvii, 299
3 Wussow Institution Quarterly of Illinois, March 31, 1913, p 71
4 Barnes Am Jour Insanity 1909, lx, 591
5 Folin, quoted by Wussow The Institution Quarterly of Illinois, March 31, 1913

TABLE 1.—DAILY DIET IN METABOLISM TESTS IN FIVE CASES OF DEMENTIA PRAECOX

Patient	Preliminary Test	Test Proper	Breakfast	Dinner	Supper
G G	Sept 30 Oct 4	Oct 4 10	Graham crackers, peanut butter, butterine, agar	Same as breakfast	Same as breakfast
A S	Dec 13-14	Dec 14 20	Rice (boiled with milk), jelly (containing English walnuts), sugared agar	Omelet (of ham, egg, cheese and milk), sugared agar	Same as breakfast
M P	Feb 4 10	Feb 10 15	(Ham - egg - cheese - milk) - omelet, graham crackers, peanut butter, butterine, agar, milk	Same as breakfast	Same as breakfast
S K	Aug 16 21	Aug 21 29	Soda crackers, peanut butter, agar sauce	Soda crackers, hamburger, agar sauce	Graham crackers, peanut butter, agar sauce
F L	Nov 13 27	Nov 27 Dec 4	Coffee, condensed milk, egg omelet, graham crackers, agar sauce	Coffee, condensed milk, hamburger, graham crackers, agar sauce	Coffee, condensed milk, peanut butter, crackers, agar sauce

A clear demarcation of the feces was many times prev
scanty stools To overcome constipation, to insure frequ
bowel movements and to obtain a sharp marking of fec
in all of the described tests It takes considerable dete
normal person to eat 10 gm of shredded agar three time
weeks, and consequently trouble was encountered in att
such an amount to disinterested insane persons Pat
induced to eat 7 gm of ground shredded agar at each m
mixed with peanut butter and butterine, and spread
number of failures followed the attempts to feed agar
other patients Patient A S took the agar when it
coated with crystal sugar The most palatable form of
yet been prepared by us and which was used in the tests o
and F L, was made by passing shredded agar through a
moistening it with water and mixing with ground ap
leaves the small pieces of agar tender enough to take awa
consistency



Urine and feces collector used in dementia praecox. metabolis
galvanized shelf which holds the feces, allowing urine to
collector in canvas case

Much difficulty was at first encountered in the collecti
feces separately or at all All tests were made on women
refused to use either bed-pan or urinal, or both If the
object to using the bed-pan and urinal, often the collecti
by the passage of both urine and feces at once into the
difficulties were all overcome by the construction of a col
shown in the illustration

Immediately after the passage of the excretions, the u
off into a bottle and the collector put into the canvas ca
to the laboratory Carmin was used as feces marker in
feces resulting from the test periods of feeding The diffi

The patient in each case was kept out of bed all day and taken daily for a walk of about two miles in the open air All of the cases were examples of dementia praecox Patients G G, A S and F L were of the chronic type Patients S K and M P were of the subsiding acute type

I am indebted to Dr H D Singer and Dr S N Clark for the following summaries of the cases

REPORT OF CASES

CHRONIC STAGE OF HEBEPHRENIC DEMENTIA PRAECOX

CASE 1.—Patient F L has been in the Kankakee State Hospital since January, 1904, and but little information is obtainable concerning her early life and the onset of the mental disorder Her mother had been "slightly insane" for from fifteen to eighteen years The patient was thought normal, married and had two children In 1901, at the age of 32, she became depressed over the death of a child and following this became gradually more restless, excited and abusive and was treated at this hospital from August to December of that year She was readmitted in January, 1904, and when received at the hospital was clear mentally, restless, irritable, experienced sense falsifications with occasional violent reaction, and felt herself under outside influence This early period of episodic restlessness, excitement and irritability apparently continued until 1907, when it was noted for the first time that she had become demented She has since been indifferent, inactive and careless and untidy in appearance, with occasional episodes of noisy restlessness lasting a few days or weeks She appears smiling, occasionally laughs and shows some stereotyped mannerisms such as suddenly pulling up her clothing The metabolism test was performed in November and December, 1913, while patient was in a period of complete quiescence

CASE 2.—Patient G G, aged 25, was admitted to the Kankakee State Hospital on Aug 22, 1912, with but scanty anamnesis The mental picture had persisted with but little change for at least four years at that time It was characterized by a general lack of interest in her surroundings with occasional outbursts of unexplained irritability, neglect of personal appearance and occasional uncleanliness It was impossible to employ her steadily and for the most part she sat smiling to herself or wandered slowly and aimlessly She appeared to be quite clear and to recognize her surroundings, but gave no connected information Speech and actions were desultory with many oddities and stereotypies There were no evidences of disease of the body organs When admitted, the patient showed hemic murmur, but it has since disappeared At the time of the test the picture was as described above and there has been no material alteration since

CASE 3.—Patient A S was admitted to the Kankakee State Hospital, Dec 9, 1910, aged 23 Very little is known of her early life, but she was considered an average child and graduated from common school at 14 She then went to work in a factory, but about two years later quietly gave it up and remained more or less indifferently at home She became more and more careless and indifferent, developed certain peculiarities, such as touching various objects, putting odds and ends in her mouth, wandering away from home, etc, and probably experienced some sense falsifications, but without acute reactions When admitted presented some physical signs suggestive of a tuberculous process in the lung, but there have been no cough, fever or loss of weight to suggest an active condition The mental picture has been one of marked absence of spontaneity, a smiling compliance with all requests, indifference to personal appearance and cleanliness, and to the situation, in spite of full clearness Answers to questions show much desultoriness of thought with some *vorbeireden* Except for a

period of a few weeks beginning in February, 1911, when she became semistuporous, had to be spoon-fed and was more or less inaccessible though without rigidity of muscles, the picture outlined above has persisted unchanged The metabolism test was made in December, 1912, while the patient was in this stationary condition

SUBSIDING STAGE OF ACUTE KATATONIC DEMENTIA PRAECOX

CASE 4.—Patient M P. was admitted to the Kankakee State Hospital, Dec 28, 1912 Her mother has suffered from some psychosis, probably dementia praecox, since 1907 The patient was born in 1882 and is described as bright intellectually, with special interest in music, but always very nervous, self-conscious and irritable She had no friends or confidants, was much alone and professed a hatred for male society During the summer of 1912 she became depressed and later more restless with many odd acts and increasing inaccessibility She also experienced auditory hallucinations In December, 1912, she passed into a state of mutism and complete inactivity with some waxy rigidity of limbs, and was in this condition when admitted The metabolism studies were undertaken in February, 1913, during this phase of katatonic stupor During the earlier part of the tests she remained entirely mute and inaccessible, but towards the end of the two weeks she began to be slightly more active and to obey simple requests The improvement continued subsequently but the patient has remained stilted and affected in speech and act, more or less indifferent to her surroundings, her friends, and her personal cleanliness, with outbursts of unmotived restless irritability and even violence, which occasionally interrupt a usual appearance of smiling indifference

CASE 5.—Patient S K was admitted to the Kankakee State Hospital, July 25, 1913, aged 20 This patient is Bohemian and it has been impossible to obtain much anamnesis She is described as an unusually bright child with no previous illness In July, 1913, she became depressed and was admitted in a condition of marked katatonic stupor with absolute mutism and entire lack of spontaneous movement, with often some waxy rigidity and command automatism Food was swallowed when placed in the mouth, but the patient paid no attention to flies or to other stimuli August 1 she spoke for the first time since admission, but did not speak again before the 15th The metabolism test was begun August 16 and continued until August 29, by which time it was possible to obtain a few answers to questions She has since improved steadily and was allowed to return to her friends Nov 16, 1913, having gained 23 pounds, though she still showed a lack of spontaneity with some vacuity and a failure to grasp the fact and nature of her illness Conversation with her was very difficult owing to language differences

Analyses of a composite of the daily food, finely ground and mixed, were made for nitrogen, sulphur, phosphorus, calcium and magnesium The urine for the entire period was well mixed and analyses made of the mixture for the different forms of nitrogen and sulphur, in addition to the analyses made on the food A composite of the total feces of the period was made in each case, and the same analyses made as on the food The methods used are the following Kjeldahl method for nitrogen, Folin's sodium-peroxid and Benedict's methods for total sulphur, Folin's methods for total sulphates and inorganic sulphates in urine, McCrudden's method for calcium and magnesium, Benedict's and Gephart's method for urea, Folin's method for ammonia, Folin-Shaffer method for uric acid and Folin's colorimetric method for creatinin The official method of the Association of Official Agricultural Chemists was used to

determine the phosphorus after its oxidation The oxidation in the case of the urine was with aqua regia, and in the case of foods and feces by ignition followed by digestion with concentrated nitric acid for four hours

The accompanying tables contain the results of the five tests

TABLE 2.—NITROGEN EXPRESSED IN GRAMS PER DAY

Type	Patient	Length of Test Days	Food	Urine	Feces	Balance
Chronic	F L	7	19 0230	7 6966	1 1616	10 1648+
	G G	6	18 2122	10 5703	1 5340	6 1078+
	A S	6	12 5496	11 8703	1 9313	1 2520—
Subsiding, acute	M P	4	16 0290	7 2058	0 7714	8 0518+
	S K	8	10 4437	7 1181	0 9619	2 3637+

TABLE 3.—DISTRIBUTION OF URINARY NITROGEN EXPRESSED IN GRAMS PER DAY

Type	Patient	Nitrogen Total	Urea	Ammonia	Creatinin	Uric Acid
Chronic	F L	7 6966	6 8366	0 1514	0 3674	0 1433
	G G	10 5703	9 5321	0 2328	0 1093	0 0722
	A S	11 8703	10 5489	0 4349	0 2489	0 0423
Subsiding, acute	M P	7 2058	5 3741	0 4426	0 1245	0 1035
	S K	7 1181	5 8038†	1 0097	0 3382	0 0738

TABLE 4.—DISTRIBUTION OF URINARY NITROGEN EXPRESSED IN PERCENTAGE OF TOTAL NITROGEN

Type	Patient	Nitrogen Total	Urea	Ammonia	Creatinin	Uric Acid
Chronic	F L	100	88 87	1 97	4 78	1 86
	G G	100	90 17	2 00	1 03	0 68
	A S	100	88 82	3 66	2 09	0 37
Subsiding, acute	M P	100	74 70	6 15	1 73	1 44
	S K	100	81 83†	14 19	4 78	1 04

† Determination was made on urine several days old, therefore the results are probably high

TABLE 5.—EXCRETION OF SULPHUR EXPRESSED IN GRAMS PER DAY

Type	Patient	Length of Test Days	Food	Urine	Feces	Balance
Chronic	L F	7	1 9620	0 7155	0 4395	0 8070+
	G G	6	1 7216	0 3143	0 4378	0 9695+
	A S	6	1 0174	0 7691	0 4210	0 1727—
Subsiding, acute	M P	4	2 1645	0 9768	0 5282	0 6895+
	S K	8	0 9756	0 5930	0 5233	0 1407—

TABLE 6.—DISTRIBUTION OF URINARY SULPHUR EXPRESSED IN GRAMS PER DAY

Type	Patient	Sulphur Total	Total Sulphate	Inorganic Sulphate	Neutral	Ethereal Sulphate
Chronic	F L	0 7155	0 6321	0 5469	0 0834	0 0852
	G G	0 3142	0 2293	0 2756	0 0847	
	A S	0 7691	0 4550	0 4421	0 3140	0 0129
Subsiding, acute	M P	0 9768	0 2858	0 2936	0 6910	
	S K	0 5930	0 4631	0 4291	0 1299	0 0340

TABLE 7.—Distribution of Urinary Sulphur Expressed in Per Cent of Total Sulphur

Type	Patient	Sulphur: Total	Total Sulphate	Inorganic Sulphate	Neutral	Ethereal Sulphate
Chronic	F L	100	88 30	76 30	11 64	11 90
	G G	100	72 99	87 72	27 02	
	A S	100	59 15	57 47	40 82	1 68
Subsiding, acute	M P	100	29 26	30 06	70 76	
	S K	100	78 12	72 39	21 91	6 08

TABLE 8.—Phosphorus Expressed in Grams Per Day

Type	Patient	Length of Test: Days	Food	Urine	Feces	Balance
Chronic	F L	7	1 2825	0 4111	0 3798	0 4916+
	G G	6	2 2200	0 5369	1 1810	0 5021+
	A S	6	1 2960	0 7193	0 5676	0 0091+
Subsiding, acute	M P	4	1 3552		0 2474	
	S K	8	0 7625	0 3393	0 6347	0 2115—

TABLE 9.—Calcium Expressed in Grams Per Day

Type	Patient	Length of Test: Days	Food	Urine	Feces	Balance
Chronic	F L	7	1 1003	0 2731	0 6978	0 1294+
	G G	6	2 4045	0 2272	1 8988	0 2785+
	A S	6	1 3068	0 2794	0 8368	0 1906+
Subsiding, acute	M P	4	1 0960	0 5954	0 3032	0 1974+
	S K	8	0 6699	0 2472	0 4314	0 0087—

TABLE 10.—Magnesium Expressed in Grams Per Day

Type	Patient	Length of Test: Days	Food	Urine	Feces	Balance
Chronic	F L	7	0 6789	0 1281	0 2875	0 2633+
	G G	6	0 6313	0 1002	0 4931	0 0380+
	A S	6	0 3521	0 1031	0 2594	0 0104—
Subsiding, acute	M P	4	0 4841	0 1161	0 1635	0 2045+
	S K	8	0 5356	0 0872	0 3302	0 1182+

TABLE 11.—Distribution of Various Elements Expressed in Relation to Nitrogen Valued at 100

Type	Patient	Food	Urine	Feces	Urine and Feces	Balance
		Sulphur				
Chronic	F L	10 32	9 29	37 82	13 04	7 94+
	G G	9 45	2 97	28 54	6 21	15 87+
	A S	8 11	6 48	21 80	8 62	13 79+
Subsiding, acute	M P	13 50	13 56	68 45	18 87	8 56+
	S K	9 34	8 33	54 42	13 82	5 95—
		Phosphorus				
Chronic	F L	6 73	5 34	32 68	8 93	4 84+
	G G	12 19	5 08	76 78	14 20	8 22+
	A S	10 32	6 06	29 40	9 32	0 73—
Subsiding, acute	M P	8 45		32 06		
	S K	7 30	4 77	66 01	12 06	8 95—
		Calcium				
Chronic	F L	5.78	3 55	60 05	10 96	1 27+
	G G	13 20	2 15	123 78	17 57	4 56+
	A S	10 41	2 35	43 34	8 08	15 23—
Subsiding, acute	M P	6 84	8 26	39 29	11 27	2 45+
	S K	6 42	3 47	44 86	8 40	0 37—
		Magnesium				
Chronic	F L	3 57	1 66	24 74	4 69	2 59+
	G G	34 66	0 95	32 14	4 90	0 62+
	A S	2 80	0 87	13 43	2 62	0 83+
Subsiding, acute	M P	3 02	1 61	21 19	3 50	2 54+
	S K	5 13	1 22	34 34	5 17	4 99+

DISCUSSION OF RESULTS

Total Nitrogen.—Table 2 indicates nothing of particular interest concerning the nitrogen distribution. With an intake of 12.5 gm. of nitrogen as in the case of Patient A. S., a negative balance is hardly to be expected. The excessive amount of nitrogen in the urine, which was responsible for this negative balance is more or less constant for this patient as shown in a previous test.[6]

Nitrogen Partition, Urea Nitrogen.—The urea nitrogen in the several cases varied from 5.37 to 9.53 gm. daily. These values are within the range of the normal urea output, but nearer the minimum than the maximum limit. When the patients are grouped according to the two classes of dementia praecox, one is immediately struck with the fact that, as far as the urea nitrogen is concerned, each class seemed to have a field of variation of its own. The chronic cases exceeded from 6.84 to 10.55 and the acute cases from 5.37 to 5.80 gm. of urea nitrogen. The same class difference is indicated in Table 4 in which the urea nitrogen is expressed in per cent. of the total urinary nitrogen. In the chronic cases it is from 88.8 to 90.2, while in the acute cases it is from 74.7 to 81.8 per cent.

Folin[7] shows that the urea output is governed to some extent by the protein intake. Folin fed 118 gm. of protein and found a urea-nitrogen output of 14.7 gm. which constituted 87.5 per cent. of the total urinary nitrogen. When he fed 6 gm. of protein, the urea-nitrogen excretion decreased to 2.2 gm., making up only 61.7 per cent. of the total urinary nitrogen. The question now arises as to whether or not in our cases the class differences in the output of urea nitrogen in grams and in per cent. were not due to a difference in the nitrogen intake. If this were true, there should be no constant difference between the two classes of patients in the number of grams of urea nitrogen excreted per gram of nitrogen ingested, and in the per cent. of urea nitrogen making up the total urinary nitrogen per gram of nitrogen ingested. That this is the case in both of the relations is shown by the following figures for Patients F. L., G. G., A. S., M. P. and S. K., respectively, 0.359, 0.523, 0.841, 0.335 and 0.556 gm. of urea nitrogen per gram of nitrogen ingested and 4.67, 4.95, 7.08, 4.66 and 7.84 per cent. of total urinary nitrogen in the form of urea nitrogen per gram of the total nitrogen intake. Since these figures do not show any definite distinction between the two classes of patients, and since it is well known that the urea nitrogen is governed to some extent by the nitrogen intake, we must conclude that in this test

6 Ross, Ellison L. Some Forms of Urinary Nitrogen Affected by the Administration of Desiccated Thyroid to Dementia Praecox Patients, THE ARCHIVES INT. MED., 1913, xii, 746.

7 Folin. Quoted by Lusk in Science of Nutrition, p. 138.

the differences in urea excretions were due to the differences in the nitrogen intake

Ammonia Nitrogen—Normally ammonia nitrogen varies from about 0 2 to 1 gm per day The amount varies in normal individuals with the diet, depending on the amount of protein ingested and on the amount of acid-forming constituents and alkali metals of the diet In pathological cases excessive ammonia output indicates an increased acid formation within the organism Von Noorden[8] states that when the ammonia nitrogen of the urine does not exceed from 8 to 10 per cent of the total nitrogen intake it cannot be said to be abnormal

In this test the ammonia nitrogen varied from 0 15 to 1 01 gm per day, entirely within the normal so far as the absolute quantities are concerned The difference between the two classes of dementia praecox in this is distinct, but not great enough to prohibit the explanation of the difference on the grounds of differences in the character of the food

In Table 4 the ammonia nitrogen is expressed in per cent of the total nitrogen of the urine Expressed in this way, ammonia nitrogen makes up from 2 0 to 14 2 per cent of the total amount in the urine Von Noorden[8] considers this manner of expressing the amount of ammonia nitrogen of no value That this is true is indicated by Folin's[7] test, cited before, in which 118 gm were fed with an output of 16 8 gm of urinary nitrogen and 0 49 gm of ammonia nitrogen, making up 3 0 per cent of the urinary nitrogen, whereas when 6 gm of protein were fed, 3 6 gm of nitrogen appeared in the urine, of which 0 42 gm, or 11 3 per cent was ammonia nitrogen In these two tests there were about equal quantities of ammonia nitrogen expressed in grams, but nearly four times as much in the second test as in the first, if measured by the per cent of the total urinary nitrogen, which made up from 60 to 90 per cent of the variable urea nitrogen It is an interesting fact, however, that in the acute cases a greater per cent of nitrogen in the form of ammonia nitrogen was excreted than in the chronic cases

If the ammonia nitrogen were expressed in per cent of the total nitrogen ingested we would have for Patients F L, G G, A S, M P, and S K, the following values 0 80, 1 28, 3 46, 2 76, and 9 71 per cent These figures show a tendency in the acute cases to the excretion of the greater amount of ammonia nitrogen The average of the values for the acute cases, 6 23 per cent, is larger than the average of any two cases in the chronic group and considerably larger than the average of all of those in the chronic group, 1 85 per cent According to Von Noorden this variation not being outside of from 8 to 10 per cent, could not be said to be abnormal

8 Von Noorden Metabolism and Practical Medicine, I, 106

Creatinin and Uric Acid Nitrogen.—No distinction can be detected between the two classes of patients by their output of creatinin and uric acid nitrogen. The amounts of creatinin and uric acid nitrogen are within the range of normal persons, according to Schaffer,[9] Hawk,[10] Neubauer, P. Munk and C. Voit,[11] Van Noorden[12] and Folin.[13]

Total Sulphur.—Table 5 indicates the distribution of the total sulphur. The intake varied from 0.97 to 2.16 gm. per day. The urinary sulphur had a greater variation, from 0.31 to 0.98 gm. per day. The fecal sulphur was remarkably constant for the two classes of patients. In the chronic class it was from 0.42 to 0.44 gm. and in the acute class from 0.52 to 0.53 gm. per day. Undoubtedly the greater part of this sulphur in the feces was in the indigestible agar, which contains about 1 per cent. of sulphur. The balances of sulphur are such as might be expected to accompany the nitrogen balances given in Table 2, except in the case of Patient S. K., who showed a positive nitrogen balance, but a negative sulphur balance. Although the character of the diet given in Table 1 is usually considered a well-balanced one, the analysis of the food and feces seems to indicate that the available sulphur occurred in an insufficient quantity in the diet of S. K.

Table 11 indicates the sulphur distribution relative to the total nitrogen distribution, the latter taken as a standard being evaluated as 100. The sulphur-nitrogen relation in the foods of the five patients varied considerably—from 8.11 to 13.50 per cent. This variation is undoubtedly due to the agar, which is poor in nitrogen and contains about 1 per cent. of sulphur.

The sulphur-nitrogen relation for the urines is interesting in the wide range of variation of from 2.97 to 13.56 per cent. The same large variation exists for the feces and for the total outgo. In the case of the urine, feces and total outgo, the sulphur-nitrogen relation of the two classes of patients materially differs. The average of all of the values or any two of the values for the chronic cases is less than the average for the acute cases. It therefore may be concluded from these tests that in the subsiding acute cases of dementia praecox there is a tendency to excrete more sulphur in proportion to nitrogen in both urine and feces than in the cases of chronic dementia praecox. Whether

9 Shaffer. Quoted by P. B. Hawk in Practical Physiological Chemistry, Ed. 3, p. 270.

10 Hawk. Quoted by P. B. Hawk in Practical Physiological Chemistry, Ed. 3, p. 266.

11 Neubauer, Munk P., and Voit, C. Quoted by Von Noorden in Metabolism and Practical Medicine, 1, p. 127.

12 Von Noorden. Metabolism and Practical Medicine, 1, p. 115.

13 Folin. Quoted by Lusk in Science of Nutrition, pp. 138, 364.

or not this tendency is outside of the limits of normal variation cannot be determined from the data available

Sulphur Partition—Total Sulphate-Sulphur—The patients in this test excreted in the urine from 0 23 to 0 63 gm of sulphur in the form of sulphates These amounts made up from 29 26 to 88 30 per cent of the total sulphur in the urine While the total sulphate-sulphur of the urine is dependent on both diet and physical condition of the subject, Hawk[14] states that normally about 2 5 gm of sulphuric acid or about 0 8 gm of sulphur are excreted per day in the sulphate form Folin[5] gives the total sulphate-sulphur output of two normal cases on a purin-free diet The first case gave an average of 1 14 gm or 94 2 per cent of the total urinary sulphur The second case gave 0 87 gm or 86 9 per cent of the total urinary sulphur Mandel and Lusk[16] found that a normal subject, on a six-day test, excreted, for every 100 gm of nitrogen ingested, an average of 6 24 gm of sulphur in the form of sulphates Our patients, taken in the order in which the cases are arranged in the tables, excreted, for every 100 gm of nitrogen ingested, 3 32, 1 26, 3 62, 1 83 and 4 45, or an average of 2 90 gm of sulphur in the form of sulphates While the above comparisons, made with the total sulphate output in these cases of dementia praecox, have not been closely comparable for reasons of differences of diet, subjects and conditions of subjects, they have shown that the patients of our test did not excrete normal amounts of sulphate sulphur

Inorganic Sulphates Tables 6 and 7 show the amount of sulphur in the form of inorganic sulphates in the urine to have varied from 0 27 to 0 55 gm per day, or from 30 06 to 87 72 per cent of the total urinary sulphur There is also a suggestion of a difference between the two classes of dementia praecox in the amounts of inorganic sulphates excreted The average of the two lowest values in the chronic cases is 0 3588 gm , or 72 59 per cent of the total urinary sulphur, and the average in all three cases is 0 4215 gm , or 73 83 per cent of the total urinary sulphur, while the average in the subsiding acute cases is 0 361 gm , or 51 22 per cent of the total There is an overlapping of values in the two classes, so that the class difference must be considered small As to the normality of the values as a whole for the inorganic sulphate sulphur output, there is little question The only data at hand for comparison are those of Folin[15] On a purin-free diet, Folin's patients averaged 1 028 gm , or 86 2 per cent , and 0 80 gm , or 78 7 per cent of the total sulphur According to these figures the chronic

14 Hawk Practical Physiological Chemistry, Ed 3, p 291

15 Folin Quoted by Lusk in Science of Nutrition, p 364

16 Mandel and Lusk Quoted by Von Noorden in Metabolism and Practical Medicine III, 613

patient's output of inorganic sulphate would be nearly normal so far as the percentage of the total sulphur is concerned, but low in absolute quantities Compared in the same way, in the acute cases the patients were below normal both in absolute weight of inorganic sulphate sulphur and in its relation to the total urinary sulphur

Neutral-Sulphur —The neutral-sulphur output of our patients is especially interesting in that it confirms Pighini's[1] findings The absolute amount varies from 0 0834 to 0 6910 gm , making up from 11 64 to 70 76 per cent of the total urinary sulphur While there is some overlapping of values in the two classes of patients, there seems to be a distinct tendency in the acute cases to the excretion of the greater amount of neutral-sulphur The average of the two highest values in the chronic class is 0 1883 gm , making up 33 92 per cent of the total sulphur, and the average of all three chronic cases 0 1607 gm , or 26 49 per cent of the total sulphur of the urine, while the average output in the acute cases was 0 4104 gm , or 46 33 per cent of the total urinary sulphur

Sherman[17] states that normally from 5 to 15 per cent of the total urinary sulphur is neutral-sulphur Von Noorden finds that the neutral sulphur normally makes up from 14 to 25 per cent of the total urinary sulphur Folin's cases averaged 5 8 and 13 1 per cent, or 0 14 and 0 10 gm , of neutral-sulphur These figures indicate that in the chronic cases an amount of neutral-sulphur very close to the limit of normal subjects was excreted, and that the acute cases were quite abnormal in this respect

Ethereal Sulphates —The data on the ethereal sulphate-sulphur output are not satisfactory or sufficient to be of any considerable importance The tendency seems to be toward a low percentage for this form of sulphur

Phosphorus —Table 8 gives the distribution of phosphorus in the food, urine, feces, and the balance The intake varied from 0 76 to 2 22 gm Sherman[18] states that in normal subjects about 1 2 gm are necessary for a phosphorus balance to result, but that a phosphorus balance has been obtained on as low as 0 90 gm per day From these facts it is evident that Patient S K did not receive sufficient phosphorus for a normal person Therefore the negative phosphorus balance in this patient was to have been expected In the urine the phosphorus seems to have been more normal in all cases Von Noorden[19] states that normally the ratio P_2O_5 N = 1 7 In our cases the ratio varies from 1 7 1 to 1 9 1

17 Sherman Chemistry of Food and Nutrition, p 268
18 Sherman Chemistry of Food and Nutrition, p 278
19 Von Noorden Metabolism and Practical Medicine, iii, 817

Calcium —The calcium distribution shown in Table 9 is relatively a normal one The intake varied from 0 6699 to 2 4045 gm per day Sherman[20] states that the normal body requires from 0 7 to 1.0 gm of CaO, or expressed in terms of Ca, from 0 5 to 0 7 gm per day The calcium intake of Patient S K was the only one which approached the minimum limit That it was her smallest calcium requirement is shown by her very small negative balance The amount of calcium in the urine varied from 0 2272 to 0 5954 gm , which, according to von Noorden,[21] is normal No difference between the two classes of patients can be detected from the absolute amounts excreted or the amounts relative to the nitrogen given in Table 2

Magnesium —Renvall[22] asserts that man normally needs 0 45 gm of magnesium per day Langworthy[23] estimates the normal bodily requirement of magnesium to be from 0 18 to 0 30 gm per day Our patients received from 0 3521 to 0 6789 gm per day According to Langworthy's standard all patients received sufficient magnesium According to Renvall's standard all but one of our patients had a sufficiency of this element and that patient had a small negative balance The magnesium content of the urines was quite constant, varying from 0 0872 to 0 1281 gm The feces content was quite variable because of the relative richness of the agar in the food The balances show nothing of importance The absolute amounts and the relative amounts of magnesium to the nitrogen in the urines, feces and balances do not show any differences between the two classes of dementia praecox

SUMMARY

Five cases of dementia praecox, three of them in a chronic and two in a subsiding acute phase of the disease, were used in the metabolism tests Over a period of from four to eight days the exact income and outgo of total nitrogen, different forms of nitrogen, total sulphur, different forms of sulphur, phosphorus, calcium and magnesium were determined

The metabolism of total nitrogen was found to be within the limits of normal with the possible exception of one case

The urea nitrogen of the urine was variable but remained normal

The ammonia nitrogen of the urine varied within the limits found in normal cases In the acute cases a tendency was exhibited to excrete more of this form of nitrogen than in the other class of patients

20 Sherman Chemistry of Food and Nutrition, p 288

21 Von Noorden Metabolism and Practical Medicine, III, 39

22 Renvall Quoted by von Noorden in Metabolism and Practical Medicine, I 428

23 Langworthy Quoted by E B Forbes in Bull Ohio Experiment Station, No 201, p 165

The amounts of creatinin and uric acid nitrogen obtained in these cases of dementia praecox coincide with the normal

The metabolism of total sulphur is considered normal, with the acute cases showing a greater sulphur excretion, both in urine and feces than the other class of patients

The total sulphate-sulphur output, according to the data on normal cases cited by authorities, was below normal

The inorganic sulphate-sulphur of the urine, according to data on normal subjects, was below normal in absolute quantities in both classes of patients, and lowest in the case of the acute class The amounts of this form of sulphur expressed in percentage of the total urinary sulphur, were about normal for the chronic class but below normal for the acute group

The urinary neutral-sulphur was practically normal for the chronic cases, but much above normal in the subsiding acute cases

The ethereal sulphate findings were negative

As to the meaning of these positive findings on the sulphur metabolism there is little question In the chronic cases there was normal total sulphur, practically normal neutral sulphur, below normal total sulphate and possibly below normal inorganic sulphate In the acute cases we have a normal, but a maximum normal, excretion of total sulphur, subnormal total sulphate, subnormal inorganic sulphate and supernormal neutral sulphur E Salkowski and Rudenko[24] state the well-known fact that an increased neutral-sulphur output at the expense of the sulphates is an indication of reduced power of tissue oxidation Therefore it must be concluded from the sulphur data that in the acute cases the patients had lost to a certain degree their normal powers of oxidation Also this is suggested in the patients in the chronic class by the subnormal sulphate output That a reduced power of oxidation should occur in cases of acute dementia praecox is not surprising, since seemingly every bodily activity is decreased That indol-acetic acid is very frequently found in the urines of dementia praecox patients[5] is evidence of a diminished power of oxidation

The findings on phosphorus, calcium and magnesium were negative

CONCLUSIONS

In these cases of dementia praecox the data show the following facts

1 The metabolism of nitrogen, phosphorus, calcium and magnesium was normal in both classes of patients

24 Salkowski, E, and Rudenko Quoted by von Noorden in Metabolism and Practical Medicine, II, 355

2 The metabolism of sulphur, except in the excessive total sulphate output and possibly the inorganic sulphate output, was normal for the chronic cases

3 The metabolism of sulphur in the acute cases was abnormal in that the excretion of total sulphur was maximum normal, the total sulphate and inorganic sulphate output were subnormal and the neutral sulphur of the urine was above normal

4 The abnormalities in the sulphur metabolism of the acute forms indicate a reduced power of metabolic oxidation The fewer and less pronounced abnormalities of sulphur metabolism in the chronic group suggest a similar, though less extensive deficiency, than in the case of the acute group

THE TITRATION OF WASSERMANN REAGENTS [*]

LOYD THOMPSON, M D
LITTLE ROCK, ARK

As the Wassermann test is considered, and should be, a quantitative reaction, the correct titration of the reagents, especially complement and amboceptor, is of the utmost importance

Wassermann,[1] in the original communication, and Noguchi,[2] as well as other writers on the subject, recommend the titration of complement and amboceptor without the addition of the other factors That is, these two factors are mixed in varying quantities with a suspension of corpuscles On incubation the smallest amount of the factor which, with a fixed quantity of the other, is required to produce complete hemolysis, is considered as one unit For the actual test twice the unit is used

This method of titration is open to objection Noguchi has pointed out that within certain limits complement and amboceptor are interchangeable Further, it is a recognized fact that on the addition of patients' serum (whether positive or negative) or antigen, more complement or amboceptor is required to produce hemolysis than when neither of these factors is added This is overcome by doubling the units of complement and amboceptor

The objection is that the worker does not know that twice the amount of complement and amboceptor which are required to produce hemolysis without serum and antigen will just produce hemolysis with them, that is, of course, if the serum is from a non-luetic patient, and the worker does not know that, when just a small amount of the complement present is bound by a slightly positive serum, enough complement may be left to produce complete hemolysis, and a weakly positive test be changed to a negative

The following method of titration of complement and amboceptor has been adopted by me in my Wassermann work

Actual test conditions are imposed on the factors throughout The human hemolytic system is employed The method of preparing the reagents has been described in a previous article [3]

* Submitted for publication Dec 8, 1913

* From the Laboratory of Clinical Diagnosis, Medical Department, University of Arkansas

1 Deutsch med Wchnschr, 1906, xxxii, 745

2 Noguchi, H The Serum Diagnosis of Syphilis, Philadelphia, 1911

3 Thompson, Loyd Oscar A Modified Wassermann, THE ARCHIVES INT MED, 1913, xi, 512

The corpuscle suspension is a 2 5 per cent solution of washed human erythrocytes The complement is a 10 per cent solution of guinea-pig serum, while the amboceptor is a solution of the serum of a rabbit, which has been immunized to human corpuscles, and diluted according to the titrated value

The corpuscle suspension is, of course, fixed and when amboceptor is titrated, it is necessary first to titrate the complement to be used against a known amboceptor, or to fix the dose of complement arbitrarily The latter is the usual method and I choose 0 5 c c as the most common unit

Fifteen tubes are required for the titration of amboceptor and eight tubes for controls The controls are the same as those used in the actual test In each of the fifteen tubes is placed 0 1 c c of a known negative inactivated serum, 0 5 c c of complement, 1 unit of antigen (diluted so that 1 unit = 0 1 c c), and the amount of physiological salt solution required to bring the total volume up to 2 5 c c , as indicated in Table 1

TABLE 1 —TITRATION OF AMBOCEPTOR

Tube	Serum	Comp	Ant	NaCl	Amboceptor	Corp	Total	Results
1	0 1	0 5	0 1	0 8	0 5 of 1 10,000	0 5	2 5	NH
2	0 1	0 5	0 1	0 7	0 6 of 1 10,000	0 5	2 5	NH
3	0 1	0 5	0 1	0 6	0 7 of 1 10,000	0 5	2 5	PH
4	0 1	0 5	0 1	0 5	0 8 of 1 10,000	0 5	2 5	PH
5	0 1	0 5	0 1	0 4	0 9 of 1 10,000	0 5	2 5	PH
6	0 1	0 5	0 1	1 2	0 1 of 1 1,000	0 5	2 5	PH
7	0 1	0 5	0 1	1 1	0 2 of 1 1,000	0 5	2 5	PH
8	0 1	0 5	0 1	1 0	0 3 of 1 1,000	0 5	2 5	H
9	0 1	0 5	0 1	0 9	0 4 of 1 1,000	0 5	2 5	H
10	0 1	0 5	0 1	0 8	0 5 of 1 1,000	0 5	2 5	H
11	0 1	0 5	0 1	0 7	0 6 of 1 1,000	0 5	2 5	H
12	0 1	0 5	0 1	0 6	0 7 of 1 1,000	0 5	2 5	H
13	0 1	0 5	0 1	0 5	0 8 of 1 1,000	0 5	2 5	H
14	0 1	0 5	0 1	0 4	0 9 of 1 1,000	0 5	2 5	H
15	0 1	0 5	0 1	0 3	1 0 of 1 1,000	0 5	2 5	H
16	0 0	0 5	0 0	0 5	1 0 of 1 1,000	0 5	2 5	H
17	0 0	0 5	0 1	0 4	1 0 of 1 1,000	0 5	2 5	H
18	0 0	0 5	0 1	1 4	0 0	0 5	2 5	NH
19	0 0	0 5	0 0	1 5	0 0	0 5	2 5	NH
20	0 0	0 0	0 1	0 9	1 0 of 1 1,000	0 5	2 5	NH
21	0 0	0 0	0 1	1 9	0 0	0 5	2 5	NH
22	0 0	0 0	0 0	1 0	1 0 of 1 1,000	0 5	2 5	NH
23	0 0	0 0	0 0	2 0	0 0	0 5	2 5	NH

The tubes are now incubated one hour in the incubator or half an hour in the water-bath at 37 C , after which the amboceptor and corpuscles are added

Tube 1 contains 0 5 c c of a 1 10,000 dilution of amboceptor and the amount is increased until Tube 15 contains 1 c c of a 1 1,000 dilution, or 0 01 c c of pure serum The incubation is continued two hours in the incubator, or one hour in the water-bath at 37 C At the end of this time the tube which contains the least amboceptor, which shows com-

plete hemolysis, is determined and this amount of amboceptor is considered as 1 unit

The titration of complement is similar except that here the amboceptor is the fixed quantity and the tubes contain increasing amounts of complement, as indicated in Table 2

TABLE 2.—TITRATION OF COMPLEMENT

Tube	Ser	Comp	Ant	NaCl	Amb	Corp	Total	Results
1	0 1	0 4	0 1	1 3	0 1	0 5	2 5	PH
2	0 1	0 5	0 1	1 2	0 1	0 5	2 5	PH
3	0 1	0 6	0 1	1 1	0 1	0 5	2 5	PH
4	0 1	0 7	0 1	1 0	0 1	0 5	2 5	H
5	0 1	0 8	0 1	0 9	0 1	0 5	2 5	H
6	0 1	0 9	0 1	0 8	0 1	0 5	2 5	H
7	0 1	1 0	0 1	0 7	0 1	0 5	2 5	H
8	0 0	1 0	0 0	0 9	0 1	0 5	2 5	H
9	0 0	1 0	0 1	0 8	0 1	0 5	2 5	H
10	0 0	1 0	0 1	0 9	0 0	0 5	2 5	NH
11	0 0	1 0	0 0	1 0	0 0	0 5	2 5	NH
12	0 0	0 0	0 1	1 8	0 1	0 5	2 5	NH
13	0 0	0 0	0 1	1 9	0 0	0 5	2 5	NH
14	0 0	0 0	0 0	1 9	0 1	0 5	2 5	NH
15	0 0	0 0	0 0	2 0	0 0	0 5	2 5	NH

In the actual performance of the test, one unit each of complement and amboceptor, and only one, is used This is all that is required, since it has been determined that, with these amounts, we have secured complete hemolysis with a known negative serum, and if, with an unknown serum, we get any inhibition of hemolysis, we know that something has bound at least part of the complement, which indicates a positive reaction

In the titration of antigen the first and most important point is to determine whether or not the antigen is hemolytic in an amount considerably in excess of the antigenic unit This is determined by adding to a corpuscle suspension gradually increasing amounts of the antigen, as indicated in Table 3

TABLE 3.—TITRATION OF ANTIGEN (HEMOLYTIC EFFECT)

Tube	Ant	Corp	NaCl	Total	Results
1	0 5 of 1 100	0 5	1 5	2 5	NH
2	0 6 of 1 100	0 5	1 4	2 5	NH
3	0 7 of 1 100	0 5	1 3	2 5	NH
4	0 8 of 1 100	0 5	1 2	2 5	NH
5	0 9 of 1 100	0 5	1 1	2 5	NH
6	0 1 of 1 10	0 5	1 9	2 5	NH
7	0 2 of 1 10	0 5	1 8	2 5	NH
8	0 3 of 1 10	0 5	1 7	2 5	NH
9	0 4 of 1 10	0 5	1 6	2 5	NH
10	0 5 of 1 10	0 5	1 5	2 5	NH
11	0 6 of 1 10	0 5	1 4	2 5	NH
12	0 7 of 1 10	0 5	1 3	2 5	NH
13	0 8 of 1 10	0 5	1 2	2 5	NH
14	0 9 of 1 10	0 5	1 1	2 5	NH
15	1 0 of 1 10	0 5	1 0	2 5	NH
16	1 5 of 1 10	0 5	0 5	2 5	PH
17	2 0 of 1 10	0 5	0 0	2 5	H

Tube 1 contains 0 5 c c of a 1 100 dilution of antigen, while Tube 17 contains 2 c c. of a 1 10 dilution, or 0 2 c c of pure antigen.

The tubes are now incubated for one hour in the water-bath at 37 C, and the one containing the largest amount of antigen in which there is no hemolysis is recorded

Working with Dr Gabby of the Psychopathic Institute at Kankakee, Ill, and Dr Nichols of the Presbyterian Hospital, Chicago, I have shown that certain antigens, which have a marked hemolytic action when added to corpuscles alone, have this action inhibited when either human or guinea-pig serum is added in small quantity (0 1 c c).

The question naturally presents itself, whether or not such an antigen would be a safe one to use, since antigen is never used in a test without the presence of patients' serum and complement I do not consider it safe, because if it is used, and the corpuscles are hemolyzed, it would not be known how much of the hemolysis is due to the amboceptor and complement, and how much to the antigen

TABLE 4.—TITRATION OF ANTIGEN (ANTICOMPLEMENTARY VALUE)

Tube	Ser	Comp	Ant	NaCl	Amb	Corp	Total	Results
1	0 1	0 5	0 5 of 1 100	0 8	0 1	0 5	2 5	H
2	0 1	0 5	0 6 of 1 100	0 7	0 1	0 5	2 5	H
3	0 1	0 5	0 7 of 1 100	0 6	0 1	0 5	2 5	H
4	0 1	0 5	0 8 of 1 100	0 5	0 1	0 5	2 5	H
5	0 1	0 5	0 9 of 1 100	0 4	0 1	0 5	2 5	H
6	0 1	0 5	0 1 of 1 10	1 2	0 1	0 5	2 5	H
7	0 1	0 5	0 2 of 1 10	1 1	0 1	0 5	2 5	H
8	0 1	0 5	0 3 of 1 10	1 0	0 1	0 5	2 5	H
9	0 1	0 5	0 4 of 1 10	0 9	0 1	0 5	2 5	H
10	0 1	0 5	0 5 of 1 10	0 8	0 1	0 5	2 5	H
11	0 1	0 5	0 6 of 1 10	0 7	0 1	0 5	2 5	H
12	0 1	0 5	0 7 of 1 10	0 6	0 1	0 5	2 5	H
13	0 1	0 5	0 8 of 1 10	0 5	0 1	0 5	2 5	H
14	0 1	0 5	0 9 of 1 10	0 4	0 1	0 5	2 5	H
15	0 1	0 5	1 0 of 1 10	0 3	0 1	0 5	2 5	PH

The second point to be determined is the anticomplementary effect of the antigen In a good antigen this effect must not be evident in an amount considerably greater than the antigenic unit A series of fifteen tubes is arranged as in Table 4, 0 1 c c of a known negative, inactivated serum is placed in each tube, one unit of previously titrated complement (usually 0 5 c c), increasing amounts of the antigen and enough salt solution to bring the total volume, when amboceptor and corpuscles are added, to 2 5 c c The tubes are now incubated for half an hour in the water-bath at 37 C, after which one unit of previously titrated amboceptor and 0 5 c c of corpuscles are added and the incubation continued for one hour The tube which contains the largest amount of antigen in which hemolysis is complete is recorded

The next and final point in the titration of antigen is the determination of the antigenic unit This titration is identical with the determi-

nation of the anticomplementary value, except that instead of using a known negative serum, a known positive serum is employed In fact, these two titrations may be, and usually are, carried out at one time After the final incubation the tubes are placed in the ice-box for a few hours, when the tube is determined which contains the smallest amount of antigen in which inhibition of hemolysis is complete

TABLE 5.—TITRATION OF ANTIGEN (ANTIGENIC UNIT)

Tube	Ser	Comp	Ant	NaCl	Amb	Corp	Total	Results
1	0 1	0 5	0 5 of 1 100	0 8	0 1	0 5	2 5	H
2	0 1	0 5	0 6 of 1 100	0 7	0 1	0 5	2 5	PH
3	0 1	0 5	0 7 of 1 100	0 6	0 1	0 5	2 5	PH
4	0 1	0 5	0 8 of 1 100	0 5	0 1	0 5	2 5	PH
5	0 1	0 5	0 9 of 1 100	0 4	0 1	0 5	2 5	PH
6	0 1	0 5	0 1 of 1 10	1 2	0 1	0 5	2 5	NH
7	0 1	0 5	0 2 of 1 10	1 1	0 1	0 5	2 5	NH
8	0 1	0 5	0 3 of 1 10	1 0	0 1	0 5	2 5	NH
9	0 1	0 5	0 4 of 1 10	0 9	0 1	0 5	2 5	NH
10	0 1	0 5	0 5 of 1 10	0 8	0 1	0 5	2 5	NH
11	0 1	0 5	0 6 of 1 10	0 7	0 1	0 5	2 5	NH
12	0 1	0 5	0 7 of 1 10	0 6	0 1	0 5	2 5	NH
13	0 1	0 5	0 8 of 1 10	0 5	0 1	0 5	2 5	NH
14	0 1	0 5	0 9 of 1 10	0 4	0 1	0 5	2 5	NH
15	0 1	0 5	1 0 of 1 10	0 3	0 1	0 5	2 5	NH

The antigenic unit is that amount of antigen which is the average of the smallest amount which will completely inhibit hemolysis with a known positive serum, and the largest amount which will cause no inhibition with a known negative serum For example, if we have found that 0 1 c c of the 1 10 dilution of antigen completely inhibits the hemolysis with a known positive serum, and 0 9 c c of antigen is the largest amount which does not inhibit hemolysis with a known negative serum, 0 5 c c is the antigenic unit

ATTEMPTS TO PRODUCE SCARLATINA IN MONKEYS *

CHARLES KRUMWIEDE, JR, MATTHIAS NICOLL, JR
AND
JOSEPHINE S PRATT
NEW YORK

Notwithstanding the numerous attempts to inoculate the lower monkeys with scarlet fever, the positive results reported, notably those of Cantacuzène[1] and Bernhardt,[2] have not been substantiated by others who have repeated the experiments of these observers (Notes 1 to 4 give the complete recent literature) Thus Landsteiner and Levaditi[3] inoculated thirty-five monkeys of five different lower species with exudate from the throat and with the blood and lymph-nodes of scarlet-fever patients, by local application to the fauces of the animals and by intravenous, intraperitoneal and subcutaneous injections of scarlet-fever products without obtaining a seemingly positive result Klimenko,[4] making use of twenty monkeys of five lower species, was equally unsuccessful, although he included among his many experimental procedures that of Bernhardt, who claims to have produced the disease in fourteen out of nineteen aimals Shortly after the publication of Cantacuzène's first papers, Dr Park inoculated six monkeys with the blood of six fresh scarlet-fever cases, including several of the malignant type The technic employed was the same as that described in Cantacuzène's report The results[5] were completely negative Our few experiments are given herewith as an additional contribution to the proof that this class of monkey is immune to the virus of scarlet fever when inoculated by any method heretofore made use of

Some of the inoculations recorded had their inception in a laboratory accident An assistant, while preparing the suspensions of streptococci for immunization of horses, sucked some of the suspension into her mouth This accident occurred Oct 24, 1912 On the morning of October 26, a beginning sore throat was noticed which became progressively worse during the day The sore throat with general symptoms of prostration continued on October 28, tingling of the skin and arms and

* From the Research Laboratory, Department of Health, New York City
* Submitted for publication Dec 10, 1913

1 Cantacuzène Compt rend Soc de biol, 1911, lxxi, 196 and 405
2 Bernhardt Deutsch med Wchnschr, 1911, xxxvii, 791
3 Landsteiner, Levaditi and Prasek Ann de l'Inst Pasteur, 1911, xxv, 754
4 Klimenko Jahrb f Kinderheilk, 1913, lxxvii, 679
5 Unpublished

chest was noticed, probably the beginning of the exanthem When seen on the morning of October 29, a general pharyngitis was present, and a typical scarlatinal rash covered the arms, chest and abdomen, the legs being comparatively free The temperature was 101 6 F, rising to 102 later in the day During the next two days the temperature fell to normal and the throat and general symptoms disappeared The subsequent course was uneventful, desquamation taking place in a typical manner As far as could be determined, there had been no contact with a case of scarlet fever

The streptococci in the suspension were the following

Scarlet fever—throat, 4 strains, 2 hemolyzing, 2 green
Mastoiditis—3 strains, 3 hemolyzing
Puerperal sepsis—3 strains, 3 hemolyzing
Pleurisy—1 strain, green
Erysipelas—1 strain, hemolyzing

EXPERIMENTS

Experiment 1.—Monkey No 593, Java, female, Nov 25, 1912, throat swabbed and sprayed with a heavy suspension of strains of streptococci previously mentioned This suspension was used in all the experiments The blood-counts taken in all will be found in the accompanying table No change in throat or tongue was observed in any animal, no inclusion bodies (Dohle), were seen in any of the smears except in Experiment 3

Observations.—The temperature, which was taken for ten days before the animal was used to determine the normal range, fluctuated between 100 and 101 4 F After inoculatioon the temperature range remained the same, except for one rise to 102 2, November 28

Experiment 2.—Dog faced baboon A, male Dec 3, 1912, throat swabbed and sprayed with the suspension

Observations.—The temperature fluctuated between 99 4 and 101 6 F for eight days preceding inoculation After inoculation the highest point was 101 8

Experiment 3.—Dog faced baboon B, female Dec 13, 1912, throat swabbed and sprayed with suspension

Observations.—The temperature before injection was only taken for three days and ranged about 101 F After injection it rose at times to 102 and twice to 102 2 F Small dot like inclusion bodies were occasionally noted, which were slightly more numerous December 4

Results.—Mixed streptococci from scarlet fever and other sources which have been grown for some months on media, caused no perceptible reaction, when applied to the throat of monkeys

Experiment 4.—Monkey 593, Macacus, female Dec 6, 1912, 2 c c of sterile 10 per cent sodium citrate solution were drawn into a syringe, which was then used to take 25 c c of blood from a child ill four and one-half days with scarlet fever The child showed a profuse rash over body, typical scarlatinal tongue and throat and enlargement of the cervical, inguinal and axillary lymph-nodes The temperature at the time was 102 F The blood was injected intraperitoneally into the monkey within a few minutes after withdrawal from the child

Observations.—Before and after injection the range of temperature fluctuated between 101 and 102 2 F December 10, 11, 12 and 13, the temperature ranged from 102 to 102 2 F At no other time did the temperature remain at this height

The noteworthy points in this experiment are that on the fourth day after the injection the temperature reached its highest point and remained high for four days On the day preceding this rise the polynuclear percentage rose and during the four days of highest temperature continued its rise with that of the total count As the monkey was menstruating at this time no weight can be placed on these changes At best they were too slight to be even suggestive of a successful inoculation

TABLE OF TOTAL NUMBER OF LEUKOCYTES AND PERCENTAGE OF POLYNUCLEARS IN EXPERIMENTS 1 TO 7

	Animal								
	593		A		B		593*		A
Days Before Inoculation	Leuk No	Polyn %	Leuk No	Polyn %	Leuk No	Polyn %	Leuk No	Polyn %	Leuk No
8	—	—	12,600	43	—	—	—	—	—
7	—	—	15,600	27	—	—	—	—	11,500
6	13,850	59	13,150	40	—	—	—	—	—
5	10,500	67	—	—	—	—	—	—	—
4	12,000	63	11,000	33	—	—	10,500	57	10,500
3	12,500	64	—	—	—	—	—	—	—
2	13,000	65	—	—	11,000	48	10,700	53	—
1	10,500	64	14,000	40	14,000	35	—	—	—
Day of Inoculation									
0	13,300	68	—	—	—	—	—	—	—
Days After Inoculation									
1	13,600	59	12,400	36	12,500	54	11,300	56	13,000
2	11,300	66	12,000	42	—	—	—	—	—
3	—	—	—	—	13,000	46	12,500	70	9,000
4	11,200	62	11,500	38	11,000	50	16,500	68	9,000
5	—	—	—	—	13,000	33	14,000	70	10,400
6	—	—	—	—	—	—	10,500	61	—
7	10,500	57	10,500	44	—	—	12,500	68	—
8	—	—	—	—	10,200	—	9,000	59	9,100
9	10,700	53	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	9,300	52	—
12	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	10,300	—	—

* Second inoculation

EXPERIMENT 5.—Dog-faced baboon A Dec 13, 1912 25 c c of blood were taken from a child of 13 five days ill with scarlatina, who showed a typical rash and scarlatinal throat and tongue The temperature at the time was 100 F The blood was injected intraperitoneally into the monkey immediatel after withdrawal from the child

Observations—The temperature reached 101 8 before injection, but as a rule fluctuated closely around 101 F After injection the range was between 101 and 102 F, giving in the chart the appearance of a slight transient rise

Result—The intraperitoneal injection of 25 c c of blood into a macacus monkey was followed by a slight rise in temperature and a coincident increase in the total and polynuclear leukocyte count, this rise, however, was slight and probably due to other causes A similar injection into a baboon was followed by no reaction

EXPERIMENT 6—Dog faced baboon 12, female March 31, 1913, swabs were smeared with the discharge from ears, throat and nose of seven children, ill from four to fifty five days with scarlet fever alone or with scarlet fever and subsequent complications The material on the swabs was washed off in sterile normal saline solution This suspension was filtered through a Berkefeld filter, the first filtrate being thrown back on the filter and saline passed through the filter after the suspension had passed through Forty c c of filtrate was obtained, which was bacterially sterile, and this was injected intraperitoneally

Observations—The temperature rose to 102 F the second day after injection The normal range for this animal was about 101 F, occasionally rising half a degree higher

EXPERIMENT 7—Dog-faced baboon 12, female April 21, 1913, swabs were collected as in the preceding experiment These were washed off in saline, giving a total of 4 c c suspension This was sprayed and swabbed on the animal's throat

Observations—The temperature did not rise above 101 6 F

Results—The intraperitoneal injection of bacteria-free material from scarlet-fever cases and the direct application to throat of the same type of material without filtration, were followed by no reaction

SUMMARY

The attempt to produce scarlet fever in monkeys and baboons by the faucial inoculation of streptococcus cultures or unfiltered exudates and discharges from scarlet-fever cases, as well as the intraperitoneal injections of filtrates of such exudates and discharges and of large quantities of blood, gave negative results

GERMAN MEASLES (RUBELLA) AN EXPERIMENTAL STUDY *

ALFRED F HESS, M D
NEW YORK

Investigations as to the nature of German measles are totally lacking. This is probably due to the fact that this disease is mild and so rarely encountered in hospital wards The only opportunity afforded for a systematic study from a laboratory standpoint is the occurrence of an epidemic in an institution where children are housed in large numbers An occasion of this kind—an epidemic attacking fifty or more children in an infant asylum—led to the present investigation, which includes a bacteriological study of the blood, inoculations of blood into monkeys with the object of producing the disease, and a cellular examination of the blood during the period of incubation of the disease

In four instances the blood was obtained for bacteriological examination In three of the cases the rash had been present for less than twenty-four hours, and in the fourth for about thirty-six hours, in two the temperature of the child was above the average, that is to say, between 102 and 103 F So that it may be said that I was able to obtain the blood in severe cases as well as at the very onset of the disease The latter circumstance would appear of particular importance when we remember that the virus of measles has been found with regularity only in the early period following the stage of exanthem Numerous cultures made on mediums containing serum and hemoglobin showed no growth whatsoever Other cultures were made by Dr Zingher, whom I wish to thank in this connection, according to the method developed by Noguchi for the cultivation of the spirochete of syphilis, the medium contains two parts of salt-free agar, one part of ascitic fluid, fresh kidney tissue, and is covered with liquid petrolatum to insure anaerobic conditions It was not found possible to obtain any growth from the inoculation of the blood in our cases. The tubes were incubated for some weeks

An attempt was made to produce this disease in monkeys Four *Macacus rhesus* monkeys were inoculated with blood obtained from four different cases In two instances the blood was from the same source as that used for purposes of cultivation In all cases the rash had existed less than twenty-four hours, in most the temperature was high when the blood was withdrawn Following is a summary of the protocols

* From the Research Laboratory, Department of Health, New York City
* Submitted for publication Jan 8, 1914

CASE 1.—Feb. 7, 1913. Child which developed rash during the night, temperature normal.

Three c.c. of citrated blood inoculated intraperitoneally into Monkey 1. The temperature was followed for four weeks. Although it frequently rose to 103 or over, there was no sudden rise or fall.

CASE 2.—March 3, 1913. Rash seen on child for first time this morning, temperature 101 F.

Five c.c. of blood obtained from right jugular vein, and inoculated intraperitoneally a few hours later into Monkey 2. The course of the temperature is shown in the accompanying chart. It will be noticed that there is a definite rise occurring on the nineteenth day after inoculation and lasting for about forty-eight hours.

CASE 3.—March 6, 1913. Child with rash for past twelve hours, temperature 101 F.

Four c.c. inoculated intraperitoneally into Monkey 3. For three weeks temperature varied between *101.1 and 102.3 F.* *No rash.*

CASE 4.—March 8, 1913. Temperature of patient 101.3 F. when blood was withdrawn. Rash had existed about twelve hours.

Eight c.c. of blood inoculated intraperitoneally into Monkey 4, cultures also taken. For the next three weeks the temperature ranged between 101.3 and 103 F. No exanthem.

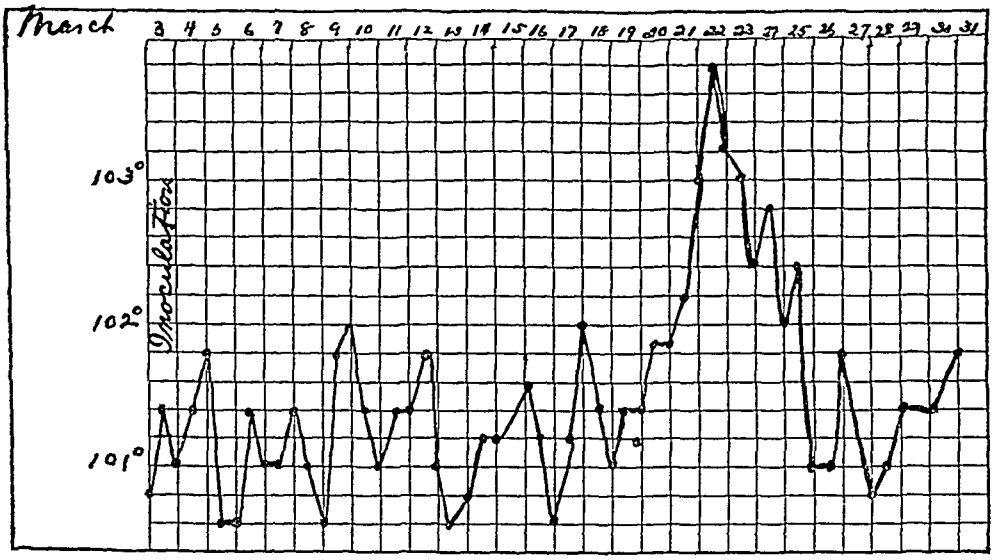


Chart of temperature of monkey (Case 2) inoculated with blood from early case of rubella.

It will be seen that in each instance from 3 to 8 c.c. of blood was inoculated intraperitoneally. In no case did the rash develop. In view of the fact however, that this appears so rarely and faintly in experimental measles, it was hardly anticipated that we would succeed in producing a rash experimentally in rubella. Our attention, therefore, was closely directed to the course of the temperature. In only one instance was there a rise in temperature, following a considerable period of incubation (see chart). In the others there was no such rise. In view of the fact that the temperature of monkeys is somewhat unstable, too great significance should not be accorded this rise of temperature. In general,

it may be stated that there is danger in placing too great reliance on a rise of temperature as the sole criterion of the production of a disease in monkeys If the rise is sudden and the period of incubation carefully controlled, however, this method of study is of value

Although there have been no cultures whatsoever made of the blood in German measles and no inoculation experiments on monkeys, there have been, as might be supposed, some previous investigations as to the number and type of leukocytes in this disease Hildebrandt and Thomas[1] found a leukopenia at the onset of the exanthem, and an increase of the lymphocytes, as well as an increase in the large Turk cells Michaels,[2] as the result of an investigation of eighty cases of rubella, also found a leukopenia in the majority of cases These studies, however, were carried out after the rash had appeared, and the only study which has taken cognizance of the blood in the earlier stages of the disease was one by Plantenga[3] Our investigation concerned itself entirely with the blood during the incubation period This was very easily carried out in the institution, for as soon as rubella developed, blood-smears were taken daily from the healthy children in the ward and were laid aside for examination in the event of some of these children developing the disease In this way, in numerous instances, consecutive blood examinations were carried out before and up to the appearance of the rash The following data show the results of these examinations, which were kindly carried out for me by Dr Jerome Zuckerman

1 H B, 2 years old Exanthem May 2

Per Cent of	April 28	April 29	April 30	May 2
Polyn	55	66	53	50
Lymph	20	22	35	35
L mono	11	5	8	10
Eosinoph	3	4	4	4
Basoph	1	3	0	1

2 L S, 3½ years old Exanthem May 2

Per Cent of	April 28	April 29	April 30	May 2
Polyn	59	51	54	41
Lymph	28	33	36	45
L Mono	5	9	9	9
Eosinoph	8	7	1	4
Basoph				1

3 S S, 5 years old Exanthem May 2

Per Cent of	April 28	April 29	April 30	May 2	May 12
Polyn	49	46	50	31	50
Lymph	35	39	40	55	34
L Mono	7	5	4	5	8
Eosinoph	5	10	5	6	4
Basoph	4	0	1	3	4

1 Hildebrandt and Thomas Ztschr f klin Med, 1906, lix, 444
2 Michaels, M Arch Pediat, 1908, xxv, 598
3 Plantenga Arch de méd d enf, 1903, vi, No 3

4 M G, 2 years old	Exanthem June 14			
Per Cent of	June 11	June 13	June 14	June 19
Polyn	43	33	34	53
Lymph	46	57	56	42
L Mono	7	7	7	5
Eosinoph	4	3	3	
5 G S, 2 years od	Exanthem June 19			
Per Cent of		June 14	June 18	June 19
Polyn		40	34	29
Lymph		51	62	63
L Mono		8	1	7
Eosinoph			3	1
6 R K, 2½ years old	Exanthem June 19			
Per Cent of		June 14	June 18	June 19
Polyn		52	35	45
Lymph		45	59	51
L Mono		3	6	4
7 W S, 2 years old	Exanthem June 19			
Per Cent of		June 14	June 18	June 19
Polyn		54	46	45
Lymph		41	52	54
Eosinoph		5	2	1

It is evident that *in almost all cases of German measles there was a definite increase in the lymphocytes even preceding the appearance of the exanthem*

It would seem that this premonitory lymphocytosis might be of value to institutions in heralding the fact that infection has taken place, and in facilitating the segregation of infected from non-infected children, and thus, perhaps, in limiting the spread of an epidemic That there is a lymphocytosis at the time of the appearance of the rash may also serve to differentiate rubella from scarlet fever, a disease with which it is sometime confounded, but which is associated with an increase of polynuclear cells in its early stage

16 West Eighty-Sixth Street

THE DIFFERENTIAL DIAGNOSIS OF THE INTESTINAL ENTAMEBAS OF MAN *

CHARLES F CRAIG, M D
Captain Medical Corps, U S Army
FORT LEAVENWORTH, KANSAS

The observations on the parasitic amebas that have been made within recent years have emphasized the importance of distinguishing between the species parasitic in the intestine of man, and it is the purpose of this contribution to give, as briefly as possible, the chief morphological points on which such a differentiation must rest, and the technic that I have found most helpful in demonstrating the morphology of these parasites In order that what follows may be clear, a brief review of our present knowledge of the intestinal entamebas of man is necessary

HISTORICAL ACCOUNT

In 1903, Schaudinn[1] described and differentiated two species of entamebas parasitic in the intestine of man One of these, the cause of the disease known as amebic dysentery, he named *Entamoeba histolytica,* while the other, a harmless commensal, he named *Entamoeba coli*

In 1905, while studying these parasites in soldiers invalided home from the Philippine Islands with dysentery, I confirmed Schaudinn's observations, and published my results in the summer of that year [2] In that paper I detailed the morphology of both species and the results of experiments on kittens with these entamebas, being the first to confirm Schaudinn's statements regarding the pathogenicity of *E histolytica,* and the non-pathogenicity of *E coli* to these animals At that time I suggested that the ameba concerned in the causation of dysentery be named *Entamoeba dysenteriae,* but as the name *dysenteriae* was afterward shown by Stiles[3] to be merely a synonym of *coli,* I adopted Schaudinn's name *histolytica* for this parasite

Hartmann,[4] in 1906, confirmed Schaudinn's classification of the intestinal entamebas of man, but for several years the distinction between *E coli* and *E histolytica* was disputed by most authorities, largely owing

* From the U S Army Central Department Laboratory
* Submitted for publication Jan 8, 1914
1 Schaudinn, F Arb a d k Gsndhtsamte, 1903, xix, 547
2 Craig, Charles F Am Med, 1905, ix, 854, 897, 937
3 Stiles C W Proc Am Public Health Assn, 1905, xxx, 292
4 Hartmann, M Arch f Protistenk, 1909, xviii, 207

to the observations of Musgrave and Clegg[5] on the amebas they secure on artificial culture-mediums and their conclusion that these ameba were identical with those found parasitic in the intestine of man, an "that all amebas are or may become pathogenic"

In 1907, Viereck[6] described a species of ameba observed in certai cases of dysentery which differed markedly from the original descriptio of *E histolytica* and which he regarded as a new species, naming i *Entamoeba tetragena* His observations were confirmed by Hartmann, Werner,[8] Darling,[9] Whitmore,[10] and myself, but further study has show that in reality *E tetragena* is identical with *E histolytica*, a portion o the life cycle of the latter parasite having been erroneously describe by Schaudinn, Hartmann and myself, as well as by others

Walker[11] was actually the first to point out that *E tetragena* i identical with *E histolytica*, and all of the work that has been accom plished by other investigators since his paper appeared has simply bee confirmatory of his conclusions, and at present all students of the subject who have had sufficient material on which to work, are agreed that tw well-defined species of entamebas occur in the intestine of man, *E his tolytica* and *E coli* The first is the cause of amebic dysentery, while th second is a harmless commensal, occurring in the intestines of a varyin percentage of individuals, in both health and disease, in almost ever part of the world

In 1905 and later, I stated[12] that the parasitic amebas of man ha not been cultivated, nor could they be cultivated, on the ordinary culture mediums employed by those who claimed to have cultivated these organ isms from the feces or other material, and this statement has been amply confirmed by nearly every subsequent investigator At present there i no adequate proof that a single species of entameba has ever been culti vated on any artificial medium A few observers still claim that the amebas that they have succeeded in cultivating are identical with the entamebas of the human intestine but their claims have been refuted by many investigators and it has been conclusively shown that the amebas so cultivated are entirely distinct from the entamebas of man, and have nothing to do with the production of dysentery

5 Musgrave, W E, and Clegg, M T Rep Bur Govt Lab, Biol Lab, Manila, 1904, No 18

6 Viereck, H. Arch f Schiffs u Tropen-Hyg, 1907, xi, 1

7 Hartmann, M Arch f Protistenk, 1912, xxiv, 163

8 Werner, H Indian Med Gaz 1909, xliv, 241

9 Darling, S T Jour Trop Med, 1912, xv, 1

10 Whitmore, E Arch f Protistenk, 1911, xxiii, 7

11 Walker, E L Philippine Jour Sc, Sec B, 1911, vi, 259

12 Craig, Charles F Am Med, 1905, ix, 854, 897, 937, Jour Infect Dis, 1908, v, 324, The Parasitic Amebae of Man, Philadelphia, 1911, p 58, Jour Infect Dis, 1913, xiii, 1

Since 1905 I have consistently maintained the specificity of *E histolytica* and *E coli*, and in my first paper, and since, have insisted that it is possible to differentiate these two species in material containing them I have also proved that neither of these parasites can be cultivated on ordinary culture-mediums, and that, while *E histolytica*, when fed to kittens or injected into the rectum, produces typical amebic dysentery and even liver abscesses, *E coli* is harmless to them Many observers have confirmed my conclusions, but the recent publication of Walker and Sellards'[13] experimental work on man with both cultural and parasitic amebas definitely proves every point for which I have contended for years They have shown conclusively that the amebas that can be cultivated from water, from the feces of man and other animals and from other sources, are incapable, when ingested, of producing the symptoms or lesions of dysentery in man, and that they are not entamebas but free-living amebas, having nothing to do with the production of dysentery and unable to live as parasites in man They have also proved beyond dispute that *E histolytica* and *E coli* are distinct species, and that when fed to man, *E histolytica* produces the symptoms and lesions of amebic dysentery, while *E coli*, though a parasitic species, is harmless

While all of the essential points covered in this paper have been previously proved by experimentation on the lower animals, the results obtained by Walker and Sellards possess the very great advantage of having been worked out on man, the natural host of *histolytica* and *coli*, and, in my opinion, settle for all time the question of the relation of parasitic and cultural amebas to disease in man

THE IMPORTANCE OF DIFFERENTIATING E HISTOLYTICA AND E COLI

In discussing the importance of differentiating between *E histolytica* and *E coli*, Walker[14] states that such differentiation is possible from a study of the morphology and life-cycle of these parasites, an opinion which I have constantly advocated, and which has been steadily gaining ground among students of the subject since the paper of Schaudinn first appeared, although some investigators, in their writings, have given the impression that such a differential diagnosis is a matter of the very greatest difficulty, only to be attempted by a protozoologist While I would be the last to state that these parasites can be differentiated by one with little or no experience in the study of intestinal protozoa, I do believe that the study of a few suitable cases of infection should enable one to differentiate the common intestinal entamebas of man Recent researches have shown that there are but two species of importance

13 Walker, E L, and Sellards, A W Philippine Jour Sc, Sec B 1913, viii, 253

14 Walker, E L Philippine Jour Sc, Sec B 1911 vi, 259

occurring in man and I am convinced that any one trained in the use of the microscope and in the study of the common bacteria and protozoa of man should have no difficulty in recognizing *E histolytica* or *E coli*

The importance of differentiating these parasites can hardly be overestimated, and attention was called to it in my first paper published in 1905 Our statistics regarding the occurrence of amebic dysentery are very largely valueless because but little effort has been made to identify the species of entameba present, numberless infections with *E coli* being mistaken for infections with *E histolytica* and diagnosed as amebic dysentery, whether symptoms were present or not In fact, some practitioners have gone so far as actually to treat for amebic dysentery every person showing an ameba in the feces, even in the absence of any effort to identify the ameba present, and very wonderful records of successful treatment of this disease have been thus built up on mistaken diagnoses The mere finding of an ameba in the feces means nothing unless the species be identified, and at present a diagnosis of entamebic dysentery based on anything else than the identification of the entameba present as *E histolytica* is not only unscientific but unjustifiable

It is now well known that *E histolytica* may live in the intestine of man for weeks, or even months, before symptoms of its presence are noticed Persons thus harboring the parasite are efficient "carriers" of the infection, and undoubtedly are largely responsible for the spread of the disease The great importance of the recognition of such infections is evident, and to recognize them it is necessary to be able to differentiate this entameba from the harmless *E coli,* so commonly present in most tropical and subtropical regions in healthy persons The importance of the discovery of these "carriers" emphasizes the great value of a routine examination of the feces of people living in regions in which entamebic dysentery is endemic, even when no symptoms of intestinal disease are present, and may be compared with the importance of the examination of the blood of both healthy and diseased persons in malarial regions in the prophylaxis of that disease Not only will the carriers of the infection thus be recognized, but treatment can be instituted when it will prove most efficient, that is, before severe lesions have been produced in the intestine

The differential diagnosis of *E histolytica* and *E coli* depends on a careful study of the morphology and life-cycle of these parasites both in the living condition and in properly stained preparations While I believe that one trained in the study of these organisms can differentiate them in the living condition, it is always best to confirm such a diagnosis by the study of a stained preparation The methods given in the following section are those I have found most useful in my own work and all of them can be followed by anyone trained in laboratory technic I have

not endeavored to give a large number of methods of staining, as I have found that it is much better to perfect oneself in one or two methods rather than to try to master all that may have been recommended

TECHNIC

The intestinal entamebas of man may be studied either in the living condition or in stained preparations, and with either method of examination a differential diagnosis between *E histolytica* and *E coli* can be made The study of the living parasites has been sadly neglected by some protozoologists, largely because of lack of material, and I am firmly of the opinion that this neglect has led to many mistakes in the interpretation of vital phenomena because of the great reliance these authorities have placed on the morphology of the stained entamebas Indeed, some investigators have gone so far as to state that these parasites should only be studied in stained preparations, a statement which appears absurd to one who has had the opportunity of studying many living preparations, for it is just as easy to differentiate these parasites in the living condition as when stained, if one has had sufficient experience

While, as I have already stated, it is always well to confirm one's species diagnosis by stained specimens, I am satisfied that in most cases a diagnosis of *E histolytica* or *E coli* can be made without recourse to staining the organisms and that this is the best and simplest method of diagnosing all types of intestinal infection with entamebas

Preparation of Living Specimens—In examining feces for living entamebas a small portion of a freshly passed stool is picked up with a platinum loop, placed on a microscopic slide, covered with a cover-glass, and gentle pressure exercised until the material is evenly spread Care should be taken not to make too thick a preparation and several should be examined before a negative result is reported I have repeatedly found a few entamebas in the ninth or tenth preparation examined, and sometimes I have had to examine as many as fifteen preparations before entamebas were discovered

The feces may be examined in the normal condition, that is, without previous purgation, or a purgative may be administered before the examination In my own experience I have obtained the best results after the administration of a purgative, especially in the demonstration of infections with *E coli*, but Walker[15] advocates the examination of stools without previous purgation, because of the greater ease with which a differential diagnosis between *E histolytica* and *E coli* may be made This has not been my experience and I would recommend the administration of a saline cathartic before the examination in all cases in which the stools are well formed If partially formed or formed stools are to be examined it is necessary to dilute a small portion of the feces with normal salt solution or the preparation will be too opaque for examination

It is always best to examine the feces immediately after passage for in such specimens the vegetative stages of the parasites, if present, are motile and therefore easily recognized Many authorities have stated that the diagnosis of an entamebic infection should never be made unless the entamebas are observed in a motile condition, but such advice is pernicious, in that it would result in over-

15 Walker, E L (With Sellards) Philippine Jour Sc, Sec B, 1913, viii, 253

looking all latent and chronic infections in which the entamebas are encysted and, of course, motionless As the recognition of the latent cases or "carriers" is of the greatest practical importance from a prophylactic point of view, it can be easily understood that such a statement might result in great harm As a matter of fact, to one who has had sufficient experience in the study of these parasites, it is just as easy to recognize a non-motile entameba as a motile one, provided degenerative changes have not occurred, and, as the cysts of entamebas persist unchanged for several hours in the feces, one may demonstrate them in material that has been collected for some time In fact, in the tropics and sub tropics, I have often observed motile entamebas in feces that had been kept at room temperature for several hours

If the feces are to be transported for any distance before the examination is made, they should be placed in a warm vacuum bottle or similar receptacle No disinfectant should be mixed with stools that are to be examined for entamebas

After preparing the specimen for examination as directed, the cover glass should be ringed with petrolatum, and the specimen examined first with a 1/6 inch objective and a 1 inch eye piece, and later with the 1/12 inch oil-immersion objective If one is experienced in the examination of feces for entamebas much time will be saved by going over the specimen with the 2/3 inch objective and 1-inch eye piece, picking out the suspicious objects, and then examining them with the higher power objectives, but considerable experience is required to recognize the entamebas with the lower-power lens mentioned

If the preparation contains motile entamebas, they are generally easily recognized even by one having little or no experience in the study of these organisms, but the non motile, precystic and encysted forms, while very distinctive to an experienced observer, may be easily confused by the inexperienced with other bodies occurring in the feces Attention to a few simple points, however, will serve to distinguish entamebas from other objects found in the feces In the resting stage these parasites are larger than pus cells or protozoa that could be confused with them, are more refractive in appearance, and are characterized by a nucleus which is small in size when compared with the nucleus of other cells, and which consists of a very refractive ring of minute granules enclosing a homogeneous substance If blood is present in the feces the red blood corpuscles are often observed within *E histolytica* and this serves at once to distinguish this parasite from other cells or protozoa

When the entamebas are encysted they are larger than the cysts of other intestinal protozoa, are spherical or oval in shape, very refractive, and possess a distinct cyst-wall From two to eight or more nuclei are present within the cyst It is comparatively easy to distinguish the cysts of *E histolytica* from those of *E coli*, as will be explained later

The use of a warm stage or an incubator for holding the microscope is not necessary unless one desires to study the life cycle of entamebas while living, when some apparatus that will keep the organisms at body temperature is absolutely necessary Much may be learned regarding their vital activities from the study of living entamebas in this manner

Preparation of Stained Specimens.—In structure the entamebas are very delicate organisms and it is undoubtedly true that any method of fixing and staining them will cause some distortion and morphological change, so that it is essential, if one desires the best results, to select staining methods that have been found to produce the least artificial change in the morphology of the parasites

In 1905 I recommended[16] the use of Wright's stain, after dry fixation, as a useful clinical method of differentiating *E histolytica* from *E coli*, and my observations regarding the use of this modification of the Romanowsky stain have since been confirmed by James,[17] who has secured excellent results by double stain-

16 Craig, Charles E Am Med, 1905, ix, 854, 897, 937
17 James, W M New York Med Jour, 1913, xcvii, 702

ing with Hastings' and Giemsa's stains While these stains, however, preceded by dry fixation, will suffice for the differential diagnosis of these parasites, much better results, as regards morphological detail, are secured by wet fixation, followed by one of the hematoxylin stains, and as some of these methods are but little more difficult of application than the modifications of the Romanowsky stain, I believe that they should be generally adopted when stained preparations of the entamebas are to be studied

To prevent alteration in morphology at any stage of fixation or staining rapid handling and the use of the process of wet fixation are necessary, as drying markedly distorts the nuclear structure of the entamebas on which a considerable part of our differential diagnosis depends

I believe that it is much better to make the smears of the material to be examined on microscopic slides, although some authorities prefer cover-glasses The slides are easier to handle and a much larger smear may be made, thus increasing the chances of finding well-stained entamebas Some form of jar should be used in which to place the various solutions used in the process of fixing and staining, to which the preparations are transferred during staining, and for this purpose I have found the Coplin staining jar, which may be obtained from any dealer in microscopical goods, an excellent one

The same directions should be followed in selecting material from the feces to be stained for entamebas as have been already described for making living preparations, but the smear must be made very rapidly and placed immediately in the fixing solution, to prevent drying, which, as remarked, will render it useless The material may be smeared in various ways but I have found that the simplest method is to place a small portion of the material to be examined near one end of the slide, and then to draw it forward over the slide with the end of another slide applied nearly at right angles to the slide containing the material A little practice will be found necessary before thin, even smears are obtained, but the method is much better than rubbing the material over the slide with a platinum loop

The character of the material examined has much to do with the success of smearing with a slide, for if much mucus be present it will be found impossible to spread it evenly in this manner, and then it will be necessary to use the platinum loop If the feces are of solid consistence, an emulsion of a portion should be made with normal salt solution, and this emulsion used for making the smears James recommends making the smears with a camel's hair brush and this is an excellent method if little mucus is present When the entamebas are few in the material to be examined, as determined by a preliminary microscopic examination, a portion may be diluted with warm normal salt solution, centrifuged, and smears then made from the sediment

The most important points to bear in mind in making the smears are that they should be as thin as possible and that they should be placed in the fixing solution immediately

Fixing the Preparations—Many solutions have been recommended for fixing entamebas, but I have found that Schaudinn's sublimate alcohol mixture answers every requirement and is a suitable mixture for use with any of the hematoxylin stains

This mixture consists of two parts of a saturated solution of mercuric chlorid in normal salt solution, and one part of absolute alcohol The mercuric chlorid solution is made by adding the mercury to boiling normal salt solution until saturation occurs This may be kept indefinitely as a stock solution

The mixture with the alcohol is made just before use and should be kept at the body temperature during the fixing process The addition of 3 per cent of glacial acetic acid improves the fixing qualities of the solution, but is not necessary

Immediately after the smears are made they are immersed in this fixing fluid, which is heated to 37 C (98 6 F), and fixed for five minutes They are then

transferred to jars containing various strengths of alcohol in the manner indicated in the following scheme

In sublimate alcohol, at 37 C for five minutes, 50 per cent alcohol for five minutes, again in 50 per cent alcohol for five minutes, 70 per cent alcohol for fifteen minutes, iodin alcohol (alcohol to which enough tincture of iodin has been added to produce a brown color) thirty minutes, 70 per cent alcohol, until the yellow color given by the iodin disappears, 95 per cent alcohol, thirty minutes, absolute alcohol, fifteen minutes, 80 per cent alcohol, five minutes, 50 per cent alcohol, ten minutes, and finally in distilled water, where they may be kept until stained

The entire process should be carried out in staining jars and only a few preparations handled at a time

While the preceding process may seem complicated, it will not be found so in practice and the time consumed will be amply repaid by the excellent results obtained The preparations must be carried rapidly from one solution to another as at no time must drying occur

Staining the Preparations—After the smears have remained in distilled water for ten minutes or longer they are ready for staining and the method selected will depend largely on the experience of the observer I have found that the ferric chlorid hematoxylin stain of Mallory gives excellent results, and this is the method I would recommend for routine work This stain and the method of applying it were first brought to my attention in the study of the entamebas by Dr W M James of Ancon, and I have found both most valuable in the differentiation of *E histolytica* and *E coli* In the following (Dr James') method of staining, as in fixing, the greatest care must be taken that the smears do not dry at any stage of the staining process

1 Having washed the preparations thoroughly in distilled water, place them on glass rods arranged over a large dish or jar, and pour over them a 10 per cent aqueous solution of ferric chlorid, so that the preparation is flooded This is the mordant and should be allowed to remain on the preparations for from ten to fifteen minutes

2 Drain the preparations, one by one, and pour over them at once a 1 per cent aqueous solution of hematoxylin, prepared by dissolving hematoxylin crystals in boiling distilled water until the mixture is cherry-red The hematoxylin solution should be freshly prepared and just warm to the hand when used As soon as the hematoxylin is added the mixture becomes black and the smear disappears from view, but enough hematoxylin should be added to render the smear just visible through the brownish-yellow solution covering it The preparations are now allowed to stain for from five to ten minutes

3 Wash the preparations thoroughly in distilled water by pouring a considerable amount over the smears or by placing them in at least two changes of distilled water contained in a staining jar

4 Decolorize in a 0 5 per cent ferric chlorid solution, the decoloration being controlled by microscopic examination When the majority of the entamebas show a well defined nucleus having a distinct membrane and containing chromatin granules, decoloration has reached the proper point and should be stopped This is accomplished by washing in distilled water If the preparations are very thick it will be necessary to use a 1 per cent solution of ferric chlorid in decolorizing

5 After decolorization, the preparations are rinsed in distilled water, washed in running tap water for fifteen minutes, dehydrated in 95 per cent and absolute alcohol, cleared in xylol, and mounted in acid-free xylol balsam If desired, the preparations may be left in water for several hours, and they should remain in each of the alcohols for at least fifteen minutes

While the preceding stain gives excellent results and is all that is necessary in routine work, more nearly perfect results, as regards the differentiation of nuclear structure, may be secured with the iron-hematoxylin method of Heidenhain This method of enabling the morphology of protozoa to be studied has never been surpassed, but it requires considerable technical ability and experience before good results are obtained

Two solutions are used in this method which is, in brief, the following
Solution 1

A 2 5 per cent solution of iron alum (Ferric ammonium sulphate)
Solution 2

Hematoxylin crystals	1 gm
Absolute alcohol	10 gm
Distilled water	*90* gm

The hematoxylin is dissolved by heat in the water and the alcohol added when the mixture is cold, after which it is placed in a dark glass bottle lightly corked with cotton, and allowed to ripen for a month or six weeks, at the end of which time it is tightly corked and ready for use

Method of Using—After fixing, as described, in sublimate-alcohol solution, the preparations are thoroughly washed in distilled water, and placed in Solution 1 for from eight to ten hours or over night, they are then rinsed in distilled water and placed in Solution 2, where they should remain for from twenty-four to forty-eight hours, after which they are rinsed, one by one, in distilled water, and placed again in Solution 1 When this is done dense black clouds come from the preparation but in from three to four minutes these almost disappear and only slightly blackish clouds are formed When this point is reached the preparations should be washed in distilled water, and placed in a jar containing tap-water The preparation should now be examined by mounting it in water with a cover-glass and using a high-power dry lens (a ⅙ or ⅛) and a 1-inch eyepiece If the entamebas show a clearly defined nucleus containing chromatin granules, *the decolorization has been sufficient, but if not, the preparation* is again washed in distilled water, placed in Solution 1 for a second or two, then mounted with a cover-glass in Solution 1 and examined under the microscope As soon as the entamebas show a well-defined nucleus, the specimen should be rinsed in distilled water, dehydrated for ten minutes each in 50, 70 and 80 per cent alcohol, in 95 per cent alcohol for fifteen minutes, and in absolute alcohol for twenty minutes, cleared in xylol and mounted in acid-free xylol balsam

While the Heidenhain stain is most reliable in experienced hands, I am of the opinion that more mistakes have been made in the interpretation of the morphology of entamebas stained in this manner than with any other stain that has been used for this purpose It is very easy to carry the process of decolorization too far, and to make the nucleus of *E coli* resemble that of *E histolytica* and I have repeatedly observed mistakes made by the best protozoologists in diagnosing between these species in preparations stained by the iron-hematoxylin method For this reason the Heidenhain method is not so generally useful as the Mallory stain, for while some experience is required, even in the use of the latter stain, it is not so difficult to manipulate and, to secure good results, requires much less experience in its use

It should be remembered, however, that no staining method is as useful in diagnosis as the examination of the living parasites, and while such methods are absolutely essential for the study of the minute morphology and reproductive cycle of the entamebas they are not essential either in the differentiation of *E coli* and *E histolytica* or in the clinical diagnosis of entamebic infection

When entamebas are well stained, by either of the methods here mentioned, the cytoplasm is of a grayish color, while the nucleus is well defined, the nuclear membrane and chromatinic granules being stained a jet black The entamebas may be confused, when stained, with other protozoa occurring in the feces or with animal or vegetable cells It is seldom difficult for one who has had experience in the study of these parasites to differentiate them from other organisms or cells, and the cysts are very characteristic, by reason of their multiple nuclei There are some vegetable cells having two or more nuclei that are often confusing to the inexperienced, being mistaken for the cysts of entamebas, but reference to the descriptions of the morphology of the true entamebic cysts should prevent such confusion Certain protozoa, as *Trichomonas hominis, Lamblia intestinalis* and *Balantidium coli* might be confused with the intestinal entamebas at certain stages in their development, but such a mistake could be made only by one having a very superficial knowledge of protozoology The *Blastocystis hominis,* a vegetable cell of uncertain origin, has frequently been mistaken for encysted entamebas, but differs greatly in morphology, only a small portion of the cell being occupied by the cytoplasm, which contains a few granules of material giving the staining reaction of chromatin

A considerable number of pages might be written describing in detail the bodies of various kinds that may be confused with the intestinal entamebas, but only experience in this line of work will prevent mistakes, and this is better gained by the study of actual preparations than by reading about differential details

THE DIFFERENTIAL DIAGNOSIS OF E HISTOLYTICA AND E COLI

The most recent observations on the intestinal entamebas have apparently proved that they pass through at least three distinct stages in their life-cycle when morphological changes occur of such importance that, unless one is well aware of them, grave errors may follow in their interpretation, and the species thus be confused This has been proved by the number of years that elapsed before it was recognized that the so-called *E tetragena* is actually but one stage in the development of *E histolytica,* this being entirely due to false interpretation of the morphology and life-cycle of the parasite The slavish insistence by some

protozoologists on the absolute value of nuclear structure in the differentiation of the entamebas has been largely responsible for the mistakes that have been made in the study of these parasites, and it is questionable whether our knowledge has been advanced or delayed by the studies of the morphologists who have devoted their energies to the study of stained entamebas, instead of the living organisms

When one remembers that slight variations in the technic of staining may cause the nucleus of *E coli* to resemble so closely that of *E histolytica* that the most expert protozoologists have been misled and have diagnosed one for the other, it will be easily understood how difficult it is for those having but a limited experience to differentiate species by the use of stained preparations

The fact remains, that after over ten years of study of the intestinal entamebas by numerous protozoologists, we are now, so far as species are concerned, just about where we were immediately after the publication of Schaudinn's paper While many new species have been described, and additions, important from a morphological point of view, have been made to our knowledge, we must admit that, at present, only two species of entameba have been actually proved to exist in the intestine of man, and these are the two species described by Schaudinn We have heard, to adapt Omar

> great argument
> About it and about but, evermore,
> Came out by the same door as in we went

It is true, however, that in properly stained preparations there are generally distinct differences in the appearance of the nucleus of *E histolytica* and *E coli*, that are sufficient to allow the experienced observer to distinguish them, while the cysts are quite characteristic for each species Moreover, it is possible to differentiate them in the living condition, and this is undoubtedly the preferable method for the clinician It should be understood that the morphology of these parasites varies greatly with the character of the feces containing them, and, therefore, with the clinical character of the infection, in the case of *E histolytica* The morphology of the latter species in cases of acute dysentery is quite different from that present in the subacute and chronic infections, so that it becomes necessary to describe the morphology of these parasites as related to the clinical course of the infection

For convenience of description I shall consider the differentiation of *E histolytica* and *E coli* during the vegetative, precystic and cystic stages of development, in both living and stained preparations, indicating in the description the clinical character of the infection present at each stage of development, in the case of *E histolytica*

THE DIFFERENTIATION OF E HISTOLYTICA AND E COLI IN THE VEGETATIVE STAGE OF DEVELOPMENT

The morphology of these parasites varies greatly during the vegetative stage of development, being most typical when the feces are liquid in character, and, in the case of *E histolytica,* when the most acute symptoms of dysentery are present When the feces are semisolid in consistence, as in acute dysentery improving under appropriate treatment, the morphology of this species is that described for the vegetative stage of *tetragena* or the so-called precystic stage, while in the acute exacerbations that occur in chronic entamebic dysentery, the nucleus of *E histolytica* is typical of the *tetragena* type of nucleus

DIFFERENTIATION OF THE LIVING ENTAMEBAS

As I have stated, *E histolytica* and *E coli* may be distinguished in the living condition and this will be found the best method in ordinary practice

Size.—It is impossible to differentiate the two species from the size of the organisms, for while *E histolytica,* in the vegetative stage of development, is generally larger than *E coli,* individuals of the latter species are observed that are fully as large as the average *histolytica,* and in the subacute types of dysentery *E histolytica* may even average smaller than *E coli* It is when acute symptoms of dysentery are present that *E histolytica* attains its largest size, measuring sometimes as much as 80 microns in the long diameter, when moving, but the average measurement is from 25 to 40 microns in diameter, when motionless The measurement of *E coli* is from 15 to 50 microns in diameter, but, as a rule, this species does not exceed 25 microns, although I have observed specimens measuring as much as 60 microns in diameter

Shape.—*E histolytica* is much more actively motile than *E coli,* and, for this reason, varies more in shape when observed in freshly passed specimens of feces While the latter is generally spherical or irregularly oval in shape, *E histolytica* may be much elongated, finger-shaped, or very irregular, owing to its marked motility Shape is of very little value, however, in the differentiation of the two species

Motility.—In freshly voided feces *E histolytica* is very actively motile when compared with *E coli,* and this is a most valuable means of distinguishing them under the condition mentioned, for I have never observed a specimen of the latter species that approached in motility the commonly observed specimens of *E histolytica* In *histolytica,* motion is generally progressive in character, while in *coli* it often consists only in the sending out and withdrawal of pseudopodia unaccompanied by progressive motility Even when progressive motion is observed in

E coli it is sluggish in character, and very easily distinguished from the motion of *E histolytica* when the two species are observed side by side In my paper published in 1905[18] I called attention to the great value of motility in distinguishing between these two parasites, and in 1908[19] stated that "the motility alone will serve to distinguish these organisms, if both be observed in freshly voided feces, for it will invariably be found that the amebas exhibiting the most active form of motility will present morphological features which show that they belong to the pathogenic species" This opinion I still hold, and it is now held by most investigators who have carefully studied the living organisms

Cytoplasm—The cytoplasm of *E histolytica* is divided into two well-defined portions, the ectoplasm and the endoplasm The distinction between the two is most marked when the parasite is moving, the glass-like ectoplasm in the advancing pseudopodium being very clearly defined from the more granular and grayish endoplasm, but even in the motionless entameba there is frequently visible a narrow rim of clear ectoplasm The ectoplasm is hyaline and glass-like in appearance while the endoplasm is granular or alveolar in structure and much less refractile Vacuoles are often absent in organisms observed in freshly voided feces, but appear in the cytoplasm as the feces become cold The clear, hyaline, homogeneous-appearing ectoplasm of this species is very characteristic and furnishes one of the most valuable differential features between it and *E coli,* as they are observed in the living condition

The cytoplasm in *E coli* is also divided into an ectoplasm and endoplasm, but the two portions are poorly defined when compared with *E histolytica* The ectoplasm of *E coli,* while distinguishable in the moving organism, does not present the clear glass-like appearance of that of *E histolytica,* but approaches very closely to the endoplasm in appearance, and gradually merges with it, instead of presenting the well-marked line of demarcation between the two portions almost always observed in *histolytica* In most instances the endoplasm of *E coli* contains one or more vacuoles, although not infrequently specimens are observed that are free from vacuoles.

To one who has had considerable experience in the study of these parasites in the living condition, the differences in the appearance of the ectoplasm and endoplasm are alone sufficient to distinguish the two species

Phagocytosis of Erythrocytes—In my early papers[20] I called attention to the facts that *E histolytica* was actively phagocytic for red blood-

18 Craig, Charles F Am Med, 1905, ix, 854, 897, 937
19 Craig, Charles F Jour Infect Dis, 1908, v, 324
20 Craig, Charles F Am Med, 1905, ix, 854, 897, 937, Internat Clin, 1905, Series 14, iv, p 242, Jour Infect Dis, 1908, v, 324

corpuscles, while *E coli* was only rarely observed to contain them, and that this was a very valuable means of distinguishing the two species This observation has been confirmed by almost every investigator who has studied these parasites in the living condition, and while the absence of erythrocytes from the cytoplasm of an entameba is of no diagnostic importance, their presence in any number in an entameba observed in feces is absolute proof that we are dealing with *E histolytica* In feces containing blood, specimens of *histolytica* are frequently observed containing erythrocytes, sometimes being absolutely filled with them, while in the same feces, specimens of *E coli* are generally entirely free from erythrocytes, and, at most, contain but one or two This fact is very useful in differentiating the species, and James, in an unpublished article, the manuscript of which he kindly sent me, says, regarding the phagocytosis of erythrocytes by these parasites

> In mixed infections I have often observed erythrocytes in varying quantities in the interior of the pathogenic organisms, but I found them rarely within the coincident *E coli*, and then only one or two at a time Absence of erythrocytes from the endoplasm is not of any particular value in differentiation, but their presence in any quantity is a certain indication of a pathogenic species

It will thus be seen that James, who has had an extensive experience in the study of these parasites on the Isthmus of Panama, confirms all that I have previously claimed regarding the importance of this phenomenon in the entameba A good clinical rule to follow, therefore, is that an entameba occurring in a dysenteric stool and containing erythrocytes should be regarded as *E histolytica*

The Nucleus—The characteristics of size, shape and division into ectoplasm and endoplasm are similar for *E histolytica* as observed in the feces from acute dysentery cases and during the acute exacerbations in the chronic stage of the disease, but this is not true of the appearance of the nucleus, in either fresh or stained preparations It was the nonrecognition of this fact that led to the description of the so-called *E tetragena* as a new species, when, in reality, one stage in the development of *E histolytica* was being observed We now know that the nuclear structure of this species may vary, at various stages in the life-history, from that typical of *histolytica,* as described by Schaudinn to that typical of *tetragena,* as described by Viereck and Hartmann, this variation being accompanied by definite changes in the clinical picture of infection with this species When the disease is in the most acute stage, *E histolytica* presents the typical *histolytica* type of nucleus, while in the subacute stage, and during the acute exacerbations of the infection in chronic cases, the *tetragena* type of nucleus is present As the latter type of nucleus approaches that of *E coli* morphologically, it is most important to differentiate it from the latter, and this is possible

in stained preparations, but in living organisms the differentiation of the two species must be made on other features than the structure of the nucleus, when the *tetragena* type is present

In acute cases of dysentery the nucleus of *E histolytica* is usually invisible, and, when visible, consists only of a very delicate, refractile ring representing the nuclear membrane, containing within it one or more refractile granules, representing the chromatin In the vast majority of instances the nucleus of this species is invisible in cases presenting acute symptoms of dysentery, thus distinguishing it from *E coli,* in which the nucleus is almost invariably visible in the living parasite

The nucleus of *E coli* consists of a well-defined ring of refractile granules, containing within it either scattered refractile granules, or a refractile mass near the center representing the karyosome It is much larger than the nucleus of *E histolytica* and can be seen with but little difficulty in either the motile or the immotile entamebas

In *E histolytica* the nucleus is generally situated near the periphery of the organism and changes its position rapidly with the movements of the ectoplasm or endoplasm, while in *E coli* the nucleus is more central in position, and, owing to the slight motility of this species, it tends to remain stationary in the endoplasm

In the subacute and early chronic cases of dysentery, however, when the nucleus of *E histolytica* is of the *tetragena* type, it is also almost always visible, having a well-defined nuclear membrane consisting of a ring of refractile granules, while within it there is considerable chromatin in the form of refractile granules or masses In my opinion, one would have to be very confident in his ability to differentiate minute morphology to assert that the nucleus of *E histolytica* could be differentiated from that of *E coli* in living organisms, when the *tetragena* type of nucleus is present At this time the differentiation of the two species must rest on other data, as I have stated

From what has been said it is evident that the differentiation of *E histolytica* and *E coli,* in the living condition and during the vegetative stage of existence, depends on several factors, the most important being motility, character of the ectoplasm and the endoplasm, and the phagocytosis of erythrocytes In making such a differentiation I believe that any entameba occurring in the intestinal tract of man possessing active motility of progressive character, having a well-differentiated ectoplasm and endoplasm, and containing erythrocytes may unhesitatingly be diagnosed as *E histolytica,* while an entameba having but sluggish motility, slight differentiation of the ectoplasm and endoplasm and no erythrocytes in the endoplasm when the feces contain blood may be correctly regarded as *E coli* I myself would not hesitate to make a

diagnosis of *E histolytica* when the feces contain entamebas with erythrocytes within them, and I believe that this is a safe clinical rule to adopt

DIFFERENTIATION IN STAINED PREPARATIONS

The differentiation of *E histolytica* and *E coli* in stained preparations, during the vegetative stage of development depends entirely on the morphology of the nucleus and on the presence of erythrocytes within the cytoplasm Neither shape nor the size neither the presence or number of vacuoles nor the distinction between ectoplasm and endoplasm is of the least value in the differentiation of the species in preparations stained in the manner described in this contribution

As in the living specimen, the presence of red blood-corpuscles within the endoplasm is sufficient to differentiate *E histolytica* from *E coli,* but very frequently the feces do not contain blood and in such instances differentiation must depend entirely on the structure of the nucleus This can be done in properly stained preparations by one experienced in the study of the protozoa but, unless this is true, it is much more safe to rely on the differences in the morphology of the living parasites in differentiating the two species during the vegetative stage of life

The Nucleus—In wet-fixed and hematoxylin-stained preparations the nucleus of *E histolytica* presents two distinct morphological types, the true *histolytica* type (Fig 1), found in the most acute cases of dysentery, and the *tetragena* type (Fig 2), found in the subacute or more chronic cases of the disease

The *histolytica* type of nucleus the species type is spherical or oval in shape in the stained preparations although if pressure has been used in making the smears the nucleus may be much distorted It is characterized by the very delicate nuclear membrane, stained black, and often not more than a line in thickness In some instances the membrane is thickened at one portion of the periphery, giving it a crescentic appearance On the inner side of the nuclear membrane very minute dots of black-stained chromatin may sometimes be observed, while at the center of the nucleus there is a very minute and delicate karyosome, usually composed of a single dot or mass of chromatin In rare instances a minute granule of chromatin, more deeply stained than the karyosome may be seen lying within the latter, forming the centriole

Within the nuclear plasma between the karyosome and the membrane a few minute dots of chromatin may be visible, lying on the nodal points of a faintly stained linin network, but very many organisms are observed in which the nucleus appears to consist only of a delicate nuclear membrane enclosing a minute karyosome, the hyaloplasm being entirely free from chromatin At this stage of development the amount of nuclear



Fig 1—Vegetative form (trophozoite) of *E histolytica*, showing typical *histolytica* type of nucleus, × 1 300 This and the following photomicrographs were taken with a No 2 eyepiece and a 1/12 oil-immersion objective All specimens were wet-fixed and stained with iron hematoxylin The photomicrographs were taken by Capt Arthur C Christy, Army Medical School, Washington D C, to whom my thanks are due for many courtesies

Fig 2—Vegetative form of *E histolytica* showing *tetragena* type of nucleus × 1,300

Fig 3—Vegetative form of *E histolytica* showing simple division One nucleus is of the typical *histolytica* type, while the other is of the *tetragena* type, × 1,300

Fig 4—Vegetative form of *E histolytica* in which the two nuclei resulting from division are of a modified *tetragena* type × 1 300

Fig 5—Vegetative form of *E histolytica* undergoing simple division The two nuclei are of the *tetragena* type × 1 300

Fig 6—Vegetative form of *E coli* Note clumps of chromatin on nuclear membane and the large compact karyosome × 1 200

chromatin is much less in *E histolytica* than in *E coli*, while the nuclear membrane and karyosome are much more delicate in appearance

In parasites undergoing simple division two nuclei may be observed, both of the *histolytica* type although rarely one of the nuclei may be of the *tetragena* type as shown in Figure 3 In the *histolytica* type of nucleus I have never observed the well-marked cyclical changes so characteristic of the *tetragena* type of nucleus

The *tetragena* type of nucleus which is characteristic of *E histolytica* during certain stages of its development, differs so markedly from the *histolytica* type that for several years entamebas showing this type of nucleus were regarded as belonging to a distinct species The nuclear membrane in stained preparations is of considerable thickness as compared with the membrane of the *histolytica* type and large clumps of chromatin are not infrequently observed on its inner surface The chromatin may be collected at the center of the nucleus forming a deeply stained solid-appearing karyosome closely resembling that of *E coli* or more frequently it has a characteristic arrangement within the nucleus, either being scattered on the inner side of the nuclear membrane or as minute deeply stained granules on the dimly stained linin network while the karyosome is comparatively large irregular or roughly spherical in shape surrounded by an unstained area and containing a well-defined centriole The latter consists of a deeply stained dot or mass of chromatin sometimes also surrounded by an unstained area

Certain well-defined cyclic changes first described by Hartmann occur in the nucleus of *E histolytica* when the *tetragena* type of nucleus is present and these changes are much more prominent in this species than in *E coli* The karyosome sometimes appears as a fine network enclosing an unstained area within which lies the centriole which may show division into two granules, the outer border of the karyosome may be bounded by an unstained area of some extent at the outer border of which the linin network is observed the boundary of the clear unstained area being formed of distinct dots of chromatin circularly arranged Again the karyosome may stain almost uniformly throughout the centriole appearing as a more deeply stained central dot or it may be invisible The linin network varies in staining qualities at times being quite distinct while at others it is almost unstainable Lying on the linin network there are generally numerous dots of chromatin which also vary in their arrangement while the inner border of the nuclear membrane is covered with minute granules of chromatin, or irregular, larger masses, quite like those occurring commonly on the nuclear membrane of *E coli* These changes in the morphology of the nucleus of *E histolytica* are certainly very characteristic of the species for while some-

what similar changes also occur in the nucleus of *E coli*, they are much less striking and offer no difficulty in the differentiation of the species

In many instances, if one searches carefully, intermediate types of nuclei between the *histolytica* and *tetragena* types may be observed in *E histolytica*, and organisms are observed containing two nuclei (Fig 4) These parasites are undergoing simple division and the two nuclei may be of the *histolytica*, *tetragena* (Fig 5) or intermediate type, or one may be of the *histolytica* type while the other is of the *tetragena* type, as already noted

In stained specimens degenerative forms of *E histolytica* are frequently observed, in which the nucleus has either wholly or partially disappeared and the cytoplasm contains threads, granules and blocks of chromatin These are the forms that have been mistaken in the past for reproduction by gemmation or budding, and that have caused so much confusion in the differentiation of the species In order to avoid such confusion it is a good working rule to regard any entameba showing chromidia within its cytoplasm as a degenerating organism which should not be considered in distinguishing between *E histolytica* and *E coli*

The nucleus of *E coli* differs from either the *histolytica* or *tetragena* type of nucleus of *E histolytica* in being much richer in chromatin, having a thicker nuclear membrane, and a larger and more compact karyosome, in which cyclical changes are not so pronounced While this is true of the vast majority of entamebas of this species, there do occur specimens of *coli* that have a nucleus very hard to distinguish from the nucleus of *E histolytica*, when that nucleus is of the *tetragena* type, so that one should study several entamebas in every specimen of feces before arriving at a conclusion regarding the presence or absence of either species

The nuclear membrane of *E coli* is quite thick and stains an intense black with hematoxylin It is even thicker than that of the *tetragena* type of *E histolytica*, and presents on its inner surface granules or, more frequently, clumps of chromatin (Fig 6) The karyosome most often appears as a deeply staining mass of chromatin, of comparatively large size, centrally situated within the nucleus, and containing no well-defined centriole In some instances a centriole may be distinguished as a more deeply staining mass of chromatin near the center of the karyosome In this species certain cyclical changes also occur in the nucleus and its appearance varies at different times but they are not so evident as in the *tetragena* type of nucleus of *E histolytica* When there is a well-defined and large karyosome there is little evidence of a linin network or of chromatin within it or on the inner surface of the nuclear membrane, but when division of the karyosome occurs the linin network is clearly visible in well-stained specimens, contains much chromatin in the form

of fine granules, and large clumps of chromatin may be observed on the inner side of the nuclear membrane (Fig 6) The nucleus of *E coli* is considerably larger than that of *E histolytica,* in the vegetative stage of development, and, while it may at times resemble the latter in morphology, the thicker nuclear membrane larger size (Fig 15), more compact karyosome, and the larger masses of chromatin on the inner side of the nuclear membrane, should serve to distinguish it from any stage in the development of the nucleus of *E histolytica*

Specimens of *E coli* are not infrequently observed undergoing simple division (Fig 8), and in stained preparations it is possible to study this process from the initial division of the centriole to the complete division of the nucleus Very frequently organisms are observed in which the karyosome consists of two deeply staining oval masses of chromatin situated near the center of the nucleus or near its periphery, others are observed in which the inner side of the nuclear membrane is covered with large, irregular clumps of chromatin, the karyosome having disappeared, and still others are observed containing two well-developed nuclei possessing the typical *coli* morphology, either situated side by side, or at some distance from one another

Degenerative forms of this species also are frequent in stained preparations and may cause confusion, but a little study of them should be sufficient to lead to a correct diagnosis regarding their nature

In summary, the following points are of most assistance in differentiating *E histolytica* from *E coli,* in stained preparations, during the vegetative stage of existence

1 The delicate nuclear membrane and minute karyosome of *E histolytica,* when the *histolytica* type of nucleus is present

2 The presence of the loose karyosome, surrounded by an unstained area, and enclosing a well-defined centriole, characteristic of the nucleus of *E histolytica,* when the *tetragena* type of nucleus is present

3 The character of the chromatin observed on the inner side of the nuclear membrane in *E histolytica,* the granules or clumps being smaller and less numerous than in *E coli*

4 The presence of erythrocytes within the cytoplasm, characteristic of *E histolytica*

The nucleus of *E coli* is characterized, in stained preparations, by the thick nuclear membrane, the large, compact karyosome in which the centriole is either invisible or ill defined, and by the large clumps of chromatin frequently observed on the inner boundary of the nuclear membrane



Fig 7—Very large cyst of *E coli*, showing the large vacuole that is frequently present in the cysts before the primary division of the nucleus Note the spindle-shaped karyosome which is frequently observed just prior to the division of this portion of the nucleus × 1,200

Fig 8—Young cysts of *E coli*, showing division of the karyosome and the thick nuclear membrane, × 1,200

Fig 9—Precystic form of *E coli* Note reduction in size and the elongation of the karyosome prior to division × 1,200

Fig 10—Early cystic stage of *E histolytica*, showing small size and typical nucleus × 1,150

Fig 11—Encysted form of *E histolytica* showing the division of the primary nucleus into two nuclei also the large chromidial masses characteristic of this species × 1,150

Fig 12—Encysted form of *E histolytica* showing the four daughter nuclei characteristic of the fully developed cyst also a large chromidial body, × 1 150

THE DIFFERENTIATION OF E HISTOLYTICA AND E COLI IN THE PRECYSTIC STAGE OF DEVELOPMENT

When the symptoms of dysentery are disappearing, that is in subacute cases there occurs a stage in the developmental cycle of *E histolytica* in which the parasite becomes markedly reduced in size (Fig 10) and the structure of the nucleus tends to assume a phase intermediate between that of the *histolytica* and *tetragena* types This is the so-called precystic stage of the parasite and, as it occurs when symptoms are slight or practically absent, it is of importance that these entamebas be distinguished from the harmless *E coli* Unfortunately, it is just at this stage in their development that it is most difficult to differentiate the two species, and if only these forms be present it is often impossible Enough typical vegetative or cystic forms are usually present however, to enable one to make the diagnosis In my opinion, it is this stage in the development of *E histolytica* that has been described by Elmassian as *E minuta*

Living Preparations—In the precystic stage of development *E histolytica* is markedly reduced in size the average measurement being from 10 to 20 microns in diameter there is less distinction between the ectoplasm and endoplasm, motility is absent or very sluggish, and a nucleus is generally visible It will thus be seen that at this stage of development this species is practically indistinguishable from *E coli,* in the living condition A careful search will generally reveal the presence of typical cysts or vegetative forms, however but unless such are present, it must be confessed that it is mere guesswork, in the vast majority of instances, to state that *E histolytica* is the parasite present

Stained Preparations—It is fully as difficult to differentiate the two species in the stained as in the living preparations, when they are in the precystic stage of development At this time the nucleus of *E histolytica* is morphologically intermediate in structure between that of the *histolytica* and *tetragena* type, the nuclear membrane being thickened, and the karyosome more compact and uniformly staining, while the centriole is often indistinguishable All of these changes cause the nucleus of this species to resemble that of *E coli,* there being a very considerable increase in the amount of nuclear chromatin preparatory to encystment I believe that it is practically impossible, in the vast majority of instances to differentiate the two species in stained preparations, at this time, but, as has been said, a careful search of the feces in such infections will generally show a few typical cysts or vegetative entamebas

Very frequently the cytoplasm of both species contains chromidia in the form of threads, granules or clumps, and it is difficult to be sure whether such organisms are degenerating or whether the appearance of the chromidia is a normal process prior to cyst formation These forms,

however, may be disregarded as they are of no value in differentiating between the two species

THE DIFFERENTIATION OF E HISTOLYTICA AND E COLI IN THE CYSTIC STAGE OF DEVELOPMENT

When the stools are semiformed or formed, following an attack of entamebic dysentery, or in the intervals between recurrences, *E histolytica* undergoes encystment, the cyst containing four nuclei (Fig 12) when fully developed In formed stools *E coli* also occurs in the encysted stage of development, the cyst of this species producing eight or more daughter nuclei (Fig 13), eight being the normal number As the cysts are the active agents of infection in both species, persons harboring them are efficient "carriers" of the infection, and hence the great importance of their discovery, especially in the prophylaxis of entamebic dysentery, which is caused by *E histolytica* Fortunately the cysts of *E histolytica* and *E coli* are quite easily recognized and differentiated both when living and when stained, and the routine examination of formed stools for these cysts in every person giving a history of diarrhea or dysentery should become as much a part of diagnostics as the examination of the sputum of persons giving a history of chronic cough The following are the most characteristic features of the morphology of the cysts in both living and stained preparations

Living Preparations —The cysts of both species are spherical or oval in shape, refractive, and present a cyst wall which varies considerably in appearance with the age of the cyst, being delicate and single in outline in the younger cysts, and thicker and having a double outline in the older cysts

Size —The cyst of *E histolytica* varies from 10 to 20 microns in diameter, the average being about 12 microns The cysts of *E coli* vary from 10 to 25 microns in diameter, the average being about 15 microns It will thus be seen that the cysts of *coli* are considerably larger than those of *histolytica*, an important differential point

Cyst Wall —The cyst wall of both species, in the newly formed cysts, consists of a delicate membrane about a line in thickness, but in the older cysts the wall becomes much thicker, and generally has a double outline The double outline is more distinct in the cysts of *E coli* than in those of *E histolytica*, but a differentiation of the species cannot be made from the character of the cyst wall

Cytoplasm —The appearance of the cytoplasm of the cysts of both species varies also with the age of the cyst In the younger cysts the cytoplasm appears granular and, in the case of *E coli* not infrequently contains included bodies as bacteria and yeast-cells, but in the older cysts these have disappeared prior to nuclear division In the early

stages of nuclear division, before and directly after the primary division of the nucleus, a large vacuole may be present in the cytoplasm in both species, but afterward this vacuole disappears in the developing cysts

Chromidia.—In the cysts of *E. histolytica* the cytoplasm very frequently contains brightly refractive, spindle-shaped, or irregular masses of chromatin, of large size, which are practically characteristic of the cysts of this species. While somewhat similar chromidial masses may rarely occur in the cysts of *E. coli*, I believe that it is justifiable to make a diagnosis of the presence of the cysts of *E. histolytica*, if these chromidial masses are present within the cyst. In the living organism they are very refractive and appear as shining masses within the cyst.

Nuclei.—The cysts of *E. histolytica*, when fully developed, are characterized by the presence of four nuclei. This number is never exceeded, in my experience, and any specimen of feces containing cysts containing no more than four nuclei may be considered as infected with this species. The nuclei appear as refractive, ring-like bodies within the cytoplasm, and it is generally necessary to focus up and down in order to bring them all into view, as they lie at different levels in the cyst. Any number of nuclei, up to and including four, may be seen in the cysts of *E. histolytica*, but never more than four.

The cysts of *E. coli* contain eight or more nuclei, when fully developed, the normal number being eight, but this is not infrequently exceeded, as cysts of this species may be observed that contain ten (Fig. 14), twelve, fourteen or even sixteen nuclei. The nuclei appear as refractive ring-like bodies, indistinguishable from those of *E. histolytica*. In my experience, any entamebic cyst occurring in the feces of man and containing more than four nuclei is the cyst of *E. coli*.

From the foregoing it is evident that in the living condition the cyst of *E. histolytica* may be distinguished from that of *E. coli* by its smaller size, the presence of the characteristic chromidial masses within the cytoplasm, and of from one to four nuclei. The cyst of *E. coli* is larger, has no chromidial masses, and may contain from one to sixteen nuclei, the normal number being eight.

Stained Preparations.—In the stained preparation, as in the living organism, the cyst of *E. histolytica* is smaller than that of *E. coli*, and the membrane is thinner and does not so often present a double outline. The characteristic differences, however, are the chromidial masses and the number of nuclei. The chromidial masses stain almost black and vary in number, only one or two being present generally. The nuclei stain intensely and are easily distinguished, but as they lie at different levels in the cysts it is necessary to focus carefully in order to be sure of the exact number. The number present, of course, determines the species of the cyst, the cysts of *E. histolytica* containing generally from two to four

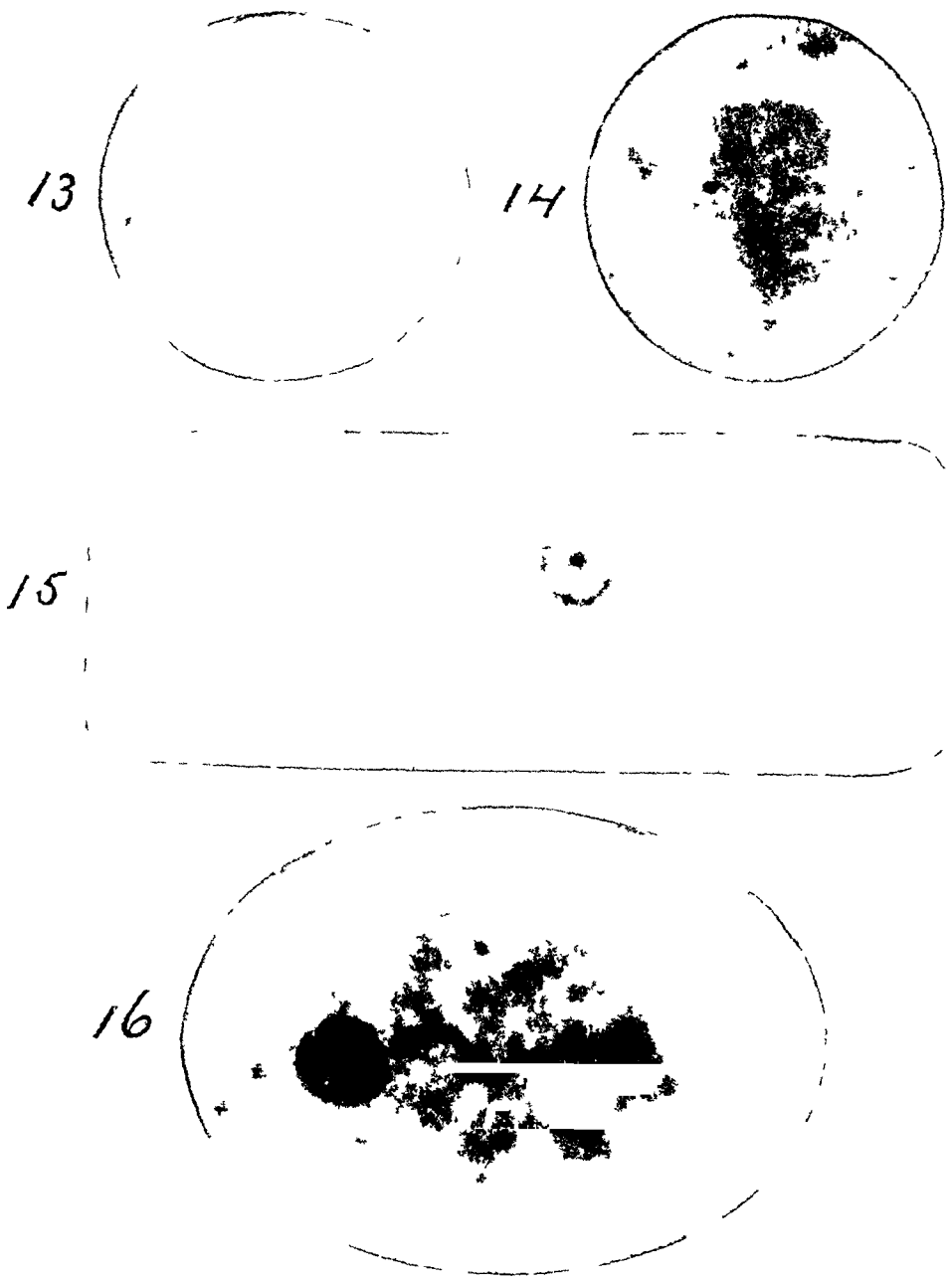


Fig 13 —Cyst of *E coli* containing eight nuclei Only six are visible in the photomicrograph because two lay at a different level in the cytoplasm and thus are out of focus Note size of cyst in comparison with cyst of *E histolytica*, × 1 200

Fig 14 —Cyst of *Entameba coli* containing ten nuclei Only five are visible, five being out of focus owing to their position in the cytoplasm Note large size of cyst × 1 200

Fig 15 —Vegetative form of *E coli* Note heavy nuclear membrane and large compact karyosome × 1.200

Fig 16 —Vegetative form of *E coli* Very large specimen showing typical nucleus × 1 200

nuclei, while those of *E coli* generally contain from two to eight nuclei, this number being sometimes exceeded

The structure of the nucleus as observed in stained preparations is quite similar, in each species, to that already described as characteristic, and, to one who has had much experience in the examination of these parasites, may alone be sufficient to differentiate the species, but this is best accomplished by attention to the number of nuclei present

In almost every specimen examined cysts may be observed that are evidently undergoing degeneration, the cytoplasm containing numerous vacuoles and threads and granules of chromatin, while no nuclei are visible These degenerate cysts have caused much confusion in the classification of entamebas, but are easily recognized and should not be considered in the differentiation of the two species under discussion The normal cysts always contain well-stained nuclei and show no chromidia in their cytoplasm, with the exception of the large chromidial masses already described as characteristic of *E histolytica*

A tabular arrangement of the chief differential points between *E histolytica* and *E coli*, in both the living condition and in stained preparations as given below, may prove of service in the diagnosis of the species It should be remembered, however, as I have stated elsewhere [21] that "a differential diagnosis of these species does not rest on the presence of a single morphological feature, but should only be made after a careful consideration of all morphological data as well as the life-cycle of the organisms investigated" While this is true, it is also true that there are certain morphological characters that are actually specific, as the presence, in any number, of erythrocytes in the cytoplasm of *E histolytica*, its four nucleated cyst, and the eight nucleated cyst of *E coli* When these are present it is an easy matter to distinguish the two species, but if only the precystic forms can be found, the differentiation is most difficult, requiring frequent examinations and the study of the life-cycle of the parasites

IMPORTANT POINTS IN THE DIFFERENTIATION OF E HISTOLYTICA AND E COLI

VEGETATIVE STAGE OF DEVELOPMENT LIVING PREPARATIONS

E histolytica	*E coli*
Actively motile	Sluggishly motile
Ectoplasm glass like Clearly differentiated from endoplasm	Ectoplasm poorly differentiated from endoplasm Not glass-like in appearance
Nucleus often indistinct or invisible	Nucleus visible and distinct
Erythrocytes present in endoplasm when stools contain blood Very characteristic	Erythrocytes absent from endoplasm when stools contain blood

21 Craig, Charles F The Parasitic Amebae of Man, Philadelphia, 1911, p 195

VEGETATIVE STAGE OF DEVELOPMENT STAINED PREPARATIONS

Delicate nuclear membrane and minute karyosome, when *histolytica* type of nucleus is present	Thick nuclear membrane and large compact karyosome (Fig 15)
Well-defined karyosome surrounded by an unstained area and containing a centriole, when the *tetragena* type of nucleus is present	Centriole generally invisible or indistinct
Smaller granules and clumps of chromatin on inner side of nuclear membrane	Large granules and clumps of chromatin on inner side of nuclear membrane (Fig 16)
Cyclical changes very marked in the nucleus when the *tetragena* type is present	Cyclical changes less marked in nucleus
Erythrocytes present in endoplasm when stools contain blood	Erythrocytes absent from endoplasm when stools contain blood

PRECYSTIC STAGE OF DEVELOPMENT LIVING AND STAINED PREPARATIONS

In both the living condition and in stained preparations the differentiation of *E histolytica* and *E coli* is most difficult in this stage of development, and often impossible The diagnosis should rest on the character of the vegetative or cystic forms that are almost invariably present together with the precystic forms

CYSTIC STAGE OF DEVELOPMENT LIVING AND STAINED PREPARATIONS

E histolytica	*E coli*
Cyst smaller	Cyst larger
Cyst wall thin Double outline less frequently observed	Cyst wall thick Double outline frequently observed
Cyst contains characteristic chromidial bodies	Cyst does not contain chromidial bodies
Cyst contains from one to four nuclei, never over four	Cyst contains from one to sixteen nuclei, the normal number being eight when the cyst is fully developed

In conclusion I would urge the value of the study of the living parasites rather than stained preparations, in the differentiation of these two species of entamebas I am convinced that for most clinicians this is the best and most practical method of distinguishing them, and that it is only those who have had a very limited experience in the study of the living entamebas who insist on the necessity of staining them in order to differentiate them The data given in this paper are the result of the study of nearly twelve hundred cases of entamebic dysentery and of hundreds of infections with *E coli,* and, in almost every case, I think it can be stated that the differentiation of *E histolytica* and *E coli* was first made on the living parasites without any special difficulty In view of this experience I am greatly in favor of the study of living organisms rather than of stained preparations in attempting to distinguish the two species There is one exception to this rule and that is in the case of the cysts I believe that the stained cysts are more readily differentiated than the living cysts, and in the search for "carriers of these bodies

better results will be obtained if stained preparations are made, as well as living preparations

The differentiation of the harmless *Entameba coli* from the pathogenic *Entameba histolytica,* and the discovery of "carriers" of the latter species, are not academic questions, but are of the most vital importance in the diagnosis, treatment and prophylaxis of dysentery, and for this reason the microscopical examination of the feces, now so much neglected by the physician, should become a routine measure in the clinical study of every patient in regions where entamebic dysentery occurs

STUDY XXI THE IMMEDIATE EFFECT OF REPEATED DOSES OF THEOBROMIN SODIUM SALICYLATE AND THEOCIN ON RENAL FUNCTION IN ACUTE EXPERIMENTAL NEPHRITIS

R FITZ, M D
BOSTON

Such authorities as Senator,[1] Osler,[2] Bradford,[3] Herrick[4] and Forschheimer[5] agree that in the treatment of acute nephritis a cautious trial with the use of diuretics may be made with safety, but that such drugs should not be given persistently unless a distinct effect is produced since large doses or the continued effect of small doses may lead to scanty excretion of urine with retention of urinary products The experimental evidence for this contention is slight

Of the diuretics in clinical use, purin derivatives are given most extensively The exact mechanism by which they produce diuresis is not altogether certain Munk[6] and Schwartz,[7] from perfusion experiments on the isolated dog's kidney injected with caffein and allied drugs, found a marked diuresis without any evidence of increased flow of blood through the kidney, and concluded that these substances had a specific effect on renal secretory functions Von Schroeder[8] and Loewi[9] confirmed these observations in normal animals, finding after injections of

* From the Medical Clinic of the Peter Bent Brigham Hospital, Boston, and Laboratory of the Theory and Practice of Physic, Harvard Medical School

* Submitted for publication Jan 16, 1914

* This is one of a series of studies on experimental cardiorenal disease Study I, Smith Boston Med and Surg Jour, 1908, clviii, 696, Study II, Christian Boston Med and Surg Jour, 1908, clix, 8, Study III, Christian Jour Am Med Assn, 1909, liii, 1792 Studies IV-XV, Christian, Smith and Walker THE ARCHIVES INT MED, 1911, viii, 468 551, Study XVI Christian and O'Hare THE ARCHIVES INT MED 1913, xi, 517, Studies XVII and XVIII, O'Hare THE ARCHIVES INT MED, 1913, xii, 49, 61 Study XIX, Christian and O'Hare, Jour Med Research, 1913, xxviii, 227, Study XX, Walker and Dawson THE ARCHIVES INT MED, 1913, xii, 171

1 Senator Nothnagel's Specielle Pathologie und Therapie, 1896, xix (1) p 64

2 Osler Practice of Medicine, 1909, p 691

3 Bradford Allbutt and Rolleston's System of Medicine 1908 iv, 607

4 Herrick Osler's Modern Medicine, 1908 vi 143

5 Forchheimer Therapeusis of Internal Diseases, 1913 iv 58

6 Munk Centralbl f die med Wissensch, 1886 xxiv 481

7 Schwartz Arch f exper Path u Pharmakol, 1899 xlii 1

8 Von Schroeder Arch f exper Path u Pharmakol 1887 xxii, 39

9 Loewi Arch f exper path u Pharmakol 1902 xlviii 410

caffein and other purin bodies an increased outflow of urine, salts and urea which they considered to be due to direct stimulation of the epithelium. Phillips and Bradford,[10] and Gottlieb and Magnus[11] supplemented these experiments by oncometer studies. They found usually after injection of diuretics of this group dilatation of the renal blood-vessels which was independent of changes in the aortic blood-pressure and which was accompanied by a marked diuresis, but occasionally diuresis occurred with little vascular dilatation or marked vascular dilatation without any additional outflow of urine. Therefore they believed that while the vasomotor phenomena were important in producing diuresis, more complex processes must be present to explain the effect of purin bodies. Fletcher, Henderson and Loewi[12] made parallel observations and drew identical conclusions. Frey,[13] however, using the oncometer, and Beco and Plumier,[14] working with a perfusion apparatus, found that the amount of urine following the injection of these diuretics was directly related to the quantity of blood going through the kidney in a given unit of time. Hence the evidence on the pharmacological action of the purin diuretics is conflicting. By the use of these drugs, however, in normal animals an increased outflow of urine and its solids occurs whether due to a specific dilating action on the blood-vessels of the kidney or to a stimulation of the renal epithelium or to a combination of both these effects.

Under pathological conditions the kidney's reaction to these drugs is varied. Hellin and Spiro[15] studied the action of caffein in rabbits poisoned with arsenic, aloin, chromium and cantharidin. Diuresis could be obtained in those nephritides in which tubular lesions predominated, but did not occur when the glomeruli were chiefly affected. Weber,[16] working on dogs, found that in chromium nephritis the kidneys were unable to excrete a concentrated urine, so that although sodium chlorid and theophyllin produced an increased outflow of fluid except in most advanced cases, yet there was no increase in the output of the solids of the urine, and in certain animals a retention. Hedinger attacked the problem from a different point of view. Schlayer and Hedinger,[17] and Schlayer and Takayasu[18] showed in experimental nephritis in rabbits that the renal vessels as tested in an oncometer were hypersensitive or hyposensitive to physiological stimuli at different stages of the disease.

10 Phillips and Bradford. Jour. Physiol., 1887, viii, 117.
11 Gottlieb and Magnus. Arch. f. exper. Path. u. Pharmakol., 1901, xlv, 223.
12 Fletcher, Henderson and Loewi. Arch. f. exper. Path. u. Pharmakol., 1905, liii, 15.
13 Frey. Arch. f. d. ges. Physiol. (Pflüger's), 1906, cxv, 175.
14 Beco and Plumier. Jour. de phys. et de path. gén., 1906, viii, 10.
15 Hellin and Spiro. Arch. f. exper. Path. u. Pharmakol., 1896, xxxviii, 368.
16 Weber. Arch. f. exper. Path. u. Pharmakol., 1906, liv, 1.
17 Schlayer and Hedinger. Deutsch. Arch. f. klin. Med., 1907, xc, 1.
18 Schlayer and Takayasu. Deutsch. Arch. f. klin. Med., 1910, xcvii, 17.

While hypersensitive the vessels dilated abnormally with a marked diuresis, while hyposensitive they no longer reacted and oliguria resulted Hedinger[19] *applied this method to animals injected with different renal* irritants, using as vascular stimuli theophyllin, digalen and digipuratum in small doses Diuresis occurred only when the renal vessels were physiologically intact MacNider[20] was unable to confirm these results entirely He observed *the renal vascular response to caffein, theobromin,* digitalen and saline solutions in dogs with uranium nephritis His animals were divided into two main groups, those anuric or practically anuric with a normal blood-pressure and normal renal vascular system which showed no diuretic reaction to the various drugs, and those with an identical blood-pressure and renal vascular response which, however, had a definite diuresis In the anuric group, the histological examination showed marked epithelial desquamation which produced an encroachment on the tubular lumen In the second group this was less distinct He concluded that mechanical obstruction to the lumen of the tubules played a part in determining the amount of urine Mosenthal and Schlayer[21] *studied the effect of repeated doses of sodium chlorid and caffein on the* amount of urine and chlorid excretion and on renal volume as recorded by an oncometer in uranium and chromate nephritis in rabbits In most advanced cases of each type of lesion neither diuretic was active, but in the early stage of chromate nephritis, sodium chlorid produced an increased outflow of urine and chlorid with renal dilatation, while caffein in repeated doses caused a diminishing amount of these substances with vascular contraction In the early stage of uranium nephritis directly opposite results were obtained These findings were explained on the assumption that in one type of lesion the functional condition was hyperesensitive to fatigue by caffein, and in the other to fatigue by chlorid Finally, Christian and O'Hare[22] gave theobromin sodium salicylate, and Walker and Dawson[23] gave theocin, caffein, potassium acetate and spartein sulphate from day to day to rabbits poisoned with uranium nitrate In most severe cases all these drugs seemed to shorten the animals' lives, but in the less severe cases theobromin sodium salicylate, at least, apparently had a beneficial action Therefore, from the literature it appears that in mild cases of acute experimental nephritis various diuretics cause an increased output of urine unless repeated rapidly enough to cause renal fatigue, and they are of possible therapeutic benefit In severe cases, however they are not pharmacologically active and are even injurious

19 Hedinger Deutsch Arch f klin Med 1910 c 305
20 MacNider Jour Pharm and Exper Therap 1912, iii 423
21 Mosenthal and Schlayer Deutsch Arch f klin Med, 1913, cxi, 217
22 Christian and O'Hare THE ARCHIVES INT MED, 1913, xi 517
23 Walker and Dawson THE ARCHIVES INT MED, 1913, xii, 171

Except for investigations regarding the total amount of urine and its constituents, no systematic studies have been reported as to the influence of diuretics on renal excretory functions under pathological conditions This paper describes a series of experiments which were made to determine the immediate effect of repeated doses of theobromin sodium salicylate and theocin on the amount of water, nitrogen, sodium chlorid and phenolsulphonephthalein eliminated during different stages of acute experimental nephritis

Rabbits were injected subcutaneously with toxic amounts of potassium bichromate or uranium nitrate in sufficient quantity to produce definite changes in renal function without rendering the animals anuric These drugs were chosen because chromium produces marked tubular destruction in the kidney, leaving the glomeruli practically intact, while uranium nitrate produces lesions in both glomeruli and tubules Thus renal function was tested under diverse pathological conditions To obtain different degrees of severity of the same type of nephritis, animals were selected at varying times after injection and renal excretion was studied in the following manner The animal to be observed was anesthetized by a subcutaneous injection of urethane (ethyl carbamate) in dosage of from 1 25 to 1 50 gm per kilogram of body-weight At the same time 2 gm of urea and 2 gm of sodium chlorid were administered in 50 c c of water by stomach-tube to insure a flow of salt and nitrogen containing fluid It was realized that these three substances in themselves were powerful diuretics, but it was believed that after their rate of excretion was once established, any marked subsequent variation during the time of these experiments was due to changes in renal function brought about by the drugs introduced Cannulas were inserted into the bladder and jugular vein according to the usual technic and the elimination of phenolsulphonephthalein, water, salt and nitrogen was followed

The phenolsulphonephthalein test was slightly modified, but was made on the whole according to Rowntree and Geraghty's[24] method for obtaining constant output of the drug At the beginning of the experiment, depending on the severity of the clinical condition of the animal, from 0 0015 to 0 006 gm of the dye in 1 c c of saline solution was injected intravenously After the color appeared, the urine during the next thirty minutes was measured in drops and diluted to 10 or 20 c c with distilled water, and the amount of phenolsulphonephthalein in an aliquot part estimated by Rowntree and Geraghty's modification of the Autenrieth-Konigsberger colorimeter Except when only traces were recovered, an amount of phenolsulphonephthalein equal to 10 per cent

24 Rowntree and Geraghty THE ARCHIVES INT MED, 1912, IX, 284

more than the total amount recovered at this time was reinjected into the vein, to be excreted during the following interval, thus allowing for the loss between observations In the few animals which were unable to excrete appreciable amounts of the drug, no further injections were made In this way the phenolsulphonephthalein concentration in the blood and tissues was kept as nearly constant as possible, and any distinct increase or decrease in the excretion was assumed to be due to changes in renal function At the end of every half hour the total amount of urine, nitrogen and salt excretion for the period was recorded The nitrogen was quantitated by Kjeldahl's method, and the chlorids by the Lutke-Martius method From these figures the concentration of nitrogen and salt in one hundred drops of urine was found After the flow of water and phenolsulphonephthalein was approximately constant for two half-hour periods, theobromin sodium salicylate and theocin in increasing dosage were injected intravenously As Hedinger[25] had found clinically that the repeated use of small amounts of diuretics often produces greater effect than single large doses, it seemed advisable to determine experimentally the action of repeated small injections of such drugs on renal function Therefore, solutions of theobromin sodium salicylate[26] and theocin sodium acetate were made containing 10 mg to the cubic centimeter of the former drug, and 5 mg to the cubic centimeter of the latter drug (corresponding to 0 3 gm of theobromin sodium salicylate and 0 15 gm of theocin if given to a man weighing 150 pounds) At first 1 c c of theobromin sodium salicylate solution was given, followed in fifteen minutes by 2 c c, yielding a total of 30 mg of the drug Then 1 c c of theocin solution was injected, and at fifteen-minute intervals 2 c c, 3 c c and 4 c c, making in all 50 mg of the drug Finally, the animal was killed and the kidneys were examined histologically after Zenker fixation and eosin-methylene blue staining The drugs were given in this order and amount for arbitrary reasons No effort was made to determine their relative efficacy of action The experiments were conducted solely to discover the immediate effect on renal function in different kinds of acute experimental nephritis of the repeated injection of two typical purin diuretics

The results have been grouped into three tables, each table representing the mean of eight experiments While each observation in general showed the same features, by averaging them in this way, any exceptional finding due to individual peculiarity of the animal was ruled out

Table 1 shows that under the conditions stated for the time observed, the excretion of fluid in normal animals diminished slightly but con-

25 Hedinger Munchen med Wchnschr, 1912, lix, 1098

26 The substance used in these experiments was the brand of theobromin sodium salicylate made by Knoll & Co and sold under the proprietary name "Diuretin" The theocin sodium acetate was made by Bayer & Co

TABLE 1.—EXPERIMENTS ON NORMAL ANIMALS

PART I NORMAL ANIMALS

Average of Rabbits I, II, III, 737 * Average weight, 1,750 gm Histological findings negative Urethane narcosis At the beginning of the experiment each animal was given 2 gm of urea and 2 gm of sodium chlorid in 50 c c of water by stomach-tube Injected intravenously with phenolsulphone phthalein

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt Injected	Remarks
		Total No Mg	Mg Per 100 Drops	Total No Mg	Mg Per 100 Drops		
1–30	180	43	24	55	31	48	
35							Reinjected with phthalein
35–65	175	54	31	48	27	46	
70							Reinjected with phthalein
70–100	164	72	44	40	24	49	Killed

* The numbers of these animals correspond to those recorded in the laboratory of Theory and Practice, Harvard Medical School

PART II NORMAL ANIMALS WITH DIURETICS

Average of Rabbits III, IV, 731, 737 Average weight, 1,750 gm Histological findings negative Urethane narcosis At the beginning of the experiment each animal was given 2 gm of urea and 2 gm of sodium chlorid in 50 c c of water by stomach tube Injected intravenously with phenol sulphonephthalein

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt Injected	Remarks
		Total No Mg	Mg Per 100 Drops	Total No Mg	Mg Per 100 Drops		
1–30	96	47	49	45	46	52	
35							Reinjected with phthalein
							1 c c of theobromin sodium salicylate
50							2 c c of theobromin sodium salicylate
35–65	150	62	41	52	34	58	
70							Reinjected with phthalein
							1 c c of theocin solution
85							2 c c of theocin solution
70–100	584	198	34	70	12	66	
105							Reinjected with phthalein
							3 c c of theocin solution
120							4 c c of theocin solution
105–135	572	188	32	61	10	64	Killed

TABLE 2.—URANIUM EXPERIMENTS

PART I ANIMALS WITHOUT DIRECTICS

Average of Rabbit 754 5 mg of uranium nitrate subcutaneously, 48-hour nephritis histologically showing marked tubular lesions

Average of Rabbit 755 3 mg of uranium nitrate subcutaneously, 24-hour nephritis histologically showing mild tubular lesions

Average of Rabbit 756 5 mg of uranium nitrate subcutaneously, 72 hour nephritis histologically showing mild tubular lesions

Average of Rabbit 757 6 mg of uranium nitrate subcutaneously in divided doses, 48 hour nephritis histologically showing advanced tubular lesions and slight glomerular lesions

Average weight, 2,000 gm Urethane narcosis At the beginning of the experiment each animal was given 2 gm of urea and 2 gm of sodium chlorid in 50 c c of water by stomach-tube Injected intravenously with phenolsulphonephthalein

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt Injected	Remarks
		Total No Mg	Mg Per 100 Drops	Total No Mg	Mg Per 100 Drops		
1–30	213	59	28	30	14	21	
35							Reinjected with phthalein
35–65	184	44	24	32	17	21	
70							Reinjected with phthalein
70–100	137	29	21	27	20	18	
105							Reinjected with phthalein
105–135	138	23	17	25	18	20	Killed

PART II ANIMALS WITH DIURETICS

Average of Rabbit 741 3 5 mg of uranium nitrate subcutaneously, 24-hour nephritis histologically showing mild tubular lesions

Average of Rabbit 730 9 mg of uranium nitrate subcutaneously in divided doses, 120-hour nephritis, histologically showing advanced tubular lesions, and slight glomerular lesions

Average of Rabbit 728 4 mg of uranium nitrate subcutaneously, 48 hour nephritis histologically showing marked tubular lesions

Average of Rabbit 729 5 mg of uranium nitrate subcutaneously in divided doses, 96-hour nephritis, histologically showing marked tubular lesions

Average weight 1,900 gm Urethane narcosis At the beginning of the experiment each animal was given 2 gm of urea and 2 gm of sodium chlorid in 50 c c of water by stomach-tube Injected intravenously with phenolsulphonephthalein

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt Injected	Remarks
		Total No Mg	Mg Per 100 Drops	Total No Mg	Mg Per 100 Drops		
1–30	176	68	39	18	10	16	
35							Reinjected with phthalein
							1 c c of theobromin sodium salicylate solution
50							2 c c of theobromin sodium salicylate solution
35–65	332	91	28	32	10	17	
70							Reinjected with phthalein
							1 c c of theocin solution
85							2 c c of theocin solution
70–100	496	137	28	38	07	18	
105							Reinjected with phthalein
							3 c c of theocin solution
120							4 c c of theocin solution
105–135	369	97	26	39	11	18	Killed

stantly, while chloiid and nitrogen excietion varied somewhat both in amount and in concentration without bearing direct relation to the amount of urine The phenolsulphonephthalein excretion remained almost unchanged When the diuretics were injected there was a pronounced increase in the amount of urine passed, paralleled by that of sodium chlorid The concentration of chlorid became lower, suggesting that the excess of salt was carried through the kidney by water The output of nitrogen was less affected by the diuresis, but increased as did that of the phenolsulphonephthalein, agreeing with Rowntree and Geraghty's[24] observations On the whole, in normal animals the immediate effect on renal function of repeated injections of theobromin sodium salicylate and theocin was beneficial This was shown by an increased output of water, chlorid, nitrogen and phenolsulphonephthalein, but by repeated dosage there was evidence of beginning renal fatigue, since the excretion of all these substances diminished during the last period of observation

As in the first part of Table 1, animals with uranium nephritis which had not received diuretics (Table 2) showed a constant diminution in the amount of urine In this series the chlorid and nitrogen decreased both in amount and in concentration The phenolsulphonephthalein remained unchanged The theobromin sodium salicylate and theocin produced a definite reaction There was a marked increase in the amount of urine paralleled by that of nitrogen and chlorid without any increase in concentration The amount of phenolsulphonephthalein, however, did not vary to an appreciable degree In the last period the evidence of renal fatigue was exaggerated The total amount of urine and salt diminished to a greater extent than in normal animals, though remaining above the initial level, while the nitrogen and phenolsulphonephthalein were unaffected Therefore, in uranium animals the immediate effect on renal function of repeated injections of theobromin sodium salicylate and theocin was not harmful There was an increased output of water, chloiid and nitrogen with an unchanged excretion of phenolsulphonephthalein The kidney was fatigued more easily than under normal conditions, as shown by the diminishing output of salt and water under the influence of repeated injections of the drugs

Table 3 shows the effect on animals poisoned with potasium bichromate and treated in similar fashion As in the previous control experiments, animals without diuretics showed a constant falling off in their salt and water excretions, although the excretion of nitiogen increased without relation to the total amount of urine The elimination of phenolsulphonephthalein was nearly constant The action of the diuretics was less marked While both salt and water were excreted in increased amounts they were proportionately less well put out than in the previous

TABLE 3.—BICHROMATE EXPERIMENTS

PART I ANIMALS WITHOUT DIURETICS

Average of Rabbit 762 20 mg of potassium bichromate subcutaneously, 72-hour nephritis histologically showing mild tubular lesions

Average of Rabbit 763 20 mg of potassium bichromate subcutaneously, 72-hour nephritis histologically showing mild tubular lesions

Average of Rabbit 764 40 mg of potassium bichromate subcutaneously, 96-hour nephritis histologically showing mild tubular lesions

Average of Rabbit 765 40 mg of potassium bichromate subcutaneously, 96 hour nephritis histologically showing well-marked tubular lesions

Average weight 2,500 gm Urethane narcosis At the beginning of the experiment each animal was given 2 gm of urea and 2 gm of sodium chlorid in 50 c c of water by stomach-tube Injected intravenously with phenolsulphonephthalein

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt Injected	Remarks
		Total No Mg	Mg Per 100 Drops	Total No Mg	Mg Per 100 Drops		
1–30 35	221	56	25	35	16	45	Reinjected with phthalein
35–65 70	219	64	29	38	17	45	Reinjected with phthalein
70–100 105	180	54	30	40	22	43	Reinjected with phthalein *
105–135	180	49	27	41	23	40*	Killed

* Represents average of three animals In one animal part of injection was lost

PART II ANIMALS WITH DIURETICS

Average of rabbit 732 20 mg of potassium bichromate subcutaneously, 24 hour nephritis histologically showing mild tubular lesions

Average of rabbit 734 20 mg of potassium bichromate subcutaneously, 72-hour nephritis histologically showing no lesions, but functionally abnormal

Average of rabbit 735 20 mg of potassium bichromate subcutaneously, 96-hour nephritis histologically showing marked tubular lesions

Average of rabbit 743 20 mg of potassium bichromate subcutaneously, 24-hour nephritis histologically showing mild tubular lesions

Average weight 1,800 gm Urethane narcosis At the beginning of the experiment each animal was given 2 gm of urea and 2 gm of sodium chlorid in 50 c c of water by stomach-tube Injected intravenously with phenolsulphonephthalein

Time Minutes from First Collected Urine	Urine Drops	Sodium Chlorid		Nitrogen		Phthalein % of Amt Injected	Remarks
		Total No Mg	Mg Per 100 Drops	Total No Mg	Mg Per 100 Drops		
1–30 35 50	192	64	33	40	21	33	Reinjected with phthalein 1 c c of theobromin sodium salicylate solution 2 c c of theobromin sodium salicylate solution
35–65 70 85	224	57	25	40	18	28	Reinjected with phthalein 1 c c of theocin solution 2 c c of theocin solution
70–100 105 120	351	96	27	45	13	24	Reinjected with phthalein 3 c c of theocin solution 4 c c of theocin solution
105–135	260	64	25	30	11	18	Killed

series There was no definite increase in nitrogen excretion, and during the last period a diminution The most striking feature, however, was the steady decrease in phenolsulphonephthalein elimination during the entire time of observation In chromate nephritis, therefore, the immediate effect of purin diuretics was to produce a slight diuresis of water and salt, to have little effect on the excretion of nitrogen and to hinder that of phenolsulphonephthalein If a large enough amount of drugs were given, the kidney was more easily fatigued than in normal or uranium animals, as shown by a greater lessening in the excretion of all the substances studied

In addition a series of experiments with cantharidin nephritis was attempted When sufficient amount of the poison was given to produce outspoken lesions, it was found that the animals became anuric and failed to react to the diuretics, confirming the observations of Hellin and Spiro,[15] Schlayer and Hedinger[17] and Schlayer and Takayasu [18] If lesser amounts were injected, the anatomical and functional conditions of the kidney were so little changed as to make the observations of no value These experiments, therefore, have been discarded

On the whole, in normal animals the immediate effect of injections of theobromin sodium salicylate and theocin was to produce an increased outflow of urine, sodium chlorid and to a less extent of nitrogen and phenolsulphonephthalein After repeated small doses of the drug were given the kidney became fatigued and excreted these substances in lesser amounts

In animals suffering from uranium nephritis of varying intensity a similar immediate response to these diuretics was obtained except that the kidney became more easily fatigued In chromium nephritis, on the other hand, while the kidney responded to the theobromin sodium salicylate and theocin by increasing the output of water and chlorid, yet the excretion of nitrogen remained almost constant and that of phenolsulphonephthalein diminished After large amounts of the drugs had been injected, the signs of renal fatigue were more pronounced than in uranium nephritis or normal animals as the water, chlorid, nitrogen and phenolsulphonephthalein excretions diminished with greater rapidity

Mosenthal and Schlayer[21] have demonstrated differences in renal fatigability to repeated injections of caffein and sodium chlorid in uranium and chromate nephritis In these experiments following repeated injections of purin diuretics the kidneys became more easily fatigued in relation to excretion of the substances studied in chromate nephritis than in uranium nephritis or normally Thus these observations are to a certain extent confirmatory of previous work

Folin, Karsner and Denis[27] have shown that the non-protein nitrogen of the blood of animals having acute toxic nephritis accumulated in proportion to the severity of the disease It was most pronounced in uranium and cantharidin nephritis and least marked in chromate nephritis Clinically, it has long been recognized as a grave prognostic sign in kidney disease Frothingham, Fitz, Folin and Denis,[28] working with rabbits poisoned with uranium nitrate, showed that the curve of nitrogen accumulation in the blood and of the excretion of phenolsulphonephthalein in the urine correspond closely Therefore, it seems reasonable to conclude that in uranium nephritis the immediate effect of theobromin sodium salicylate and theocin was beneficial for by the use of these drugs the outflow of water, sodium chlorid and nitrogen was increased, thereby tending to prevent nitrogenous accumulation in the blood In chromate nephritis, however, the immediate effect of these diuretics was harmful, since, although they increased the outflow of water and chlorid, the nitrogen excretion was unaffected and the phenolsulphonephthalein elimination was diminished In each nephritis repeated injections of theobromin sodium salicylate and theocin to the point of fatigue of the kidney was harmful to the renal function by producing a diminution in the total amount of urine, chlorid, and to a less degree, of phenolsulphonephthalein and nitrogen excreted

CONCLUSIONS

1 The immediate effect of repeated small doses of theobromin sodium salicylate and theocin on renal function in acute experimental uranium nephritis of different degrees of severity is beneficial since they cause an increased output of urine, chlorid and nitrogen and do not diminish the excretion of phenolsulphonephthalein

2 The immediate effect of repeated small doses of theobromin sodium salicylate and theocin on renal function in acute experimental bichromate nephritis of different degrees of intensity is harmful, since although an increased output of urine and chlorid occurs, the excretion of nitrogen is unaffected and that of phenolsulphonephthalein is diminished

3 The repeated injection of small doses of theobromin sodium salicylate and theocin in sufficient amount produces a condition of renal fatigue in which the kidney is no longer able to react This is most easily obtained in bichromate nephritis

4 Various types of acute experimental nephritis respond differently to repeated injections of theobromin sodium salicylate and theocin both in regard to immediate changes in the excretion of chlorid, nitrogen and

27 Folin, Karsner and Denis Jour Exper Med, 1912, xvi 789

28 Frothingham, Fitz Folin and Denis THE ARCHIVES INT MED, 1913. xii, 245

phenolsulphonephthalein and in regard to the degree of renal fatigue which is induced

5 These experiments do not include observations on nephritis of the most extreme grade of severity In the few studies made on animals which had the different kinds of lesions and were anuric, the diuretics were not active in provoking an increased output of either water, chlorid, nitrogen or phenolsulphonephthalein

Peter Bent Brigham Hospital

THREE CASES OF ADAMS-STOKES SYNDROME WITH HISTOLOGICAL FINDINGS*

ADELE OPPENHEIMER A.M. AND B. S. OPPENHEIMER, M.D.
NEW YORK

Although it is established that a complete transverse lesion of the stem of the auriculoventricular conducting system or of its two main branches results in auriculoventricular dissociation, and although a few years ago the problem of heart-block seemed almost settled by the discovery of lesions in the conducting system, it is now apparent that the solution of this problem is not so simple as it appeared at that time. There are cases on record in which heart-block was proved beyond a doubt but still no lesions in the hearts adequately explaining the failure of the conduction of impulses from auricle to ventricle were to be found.†

The three cases to be presented show three very different histological conditions in the conducting system: and it is somewhat unfortunate that the clinical data do not give us such definite information of the mechanism of the heart as can be positively correlated with these post-mortem findings.

Three hearts from patients with a clinical history suggesting heart-block were kindly entrusted to us for microscopic examination; two by Dr. MacCallum and one by Dr. Bishop.

HEART 1.—*Case Report.*—The case report has been given by Dr. Bishop.[1] The patient, a watchman, aged 75, was admitted to Lincoln Hospital Oct. 22, 1908. His family history was negative. He had had measles, whooping cough and small-pox when a child. Later in life he had had muscular rheumatism and a sunstroke. He had drunk whisky moderately. Syphilitic history was denied. The patient was described as having a slow pulse, vertiginous and syncopal attacks and rapid venous pulse. The radial artery was not thickened even to the degree that would be expected in a patient of his age. A polygraphic tracing was published in the original article.

Macroscopic Examination of the Heart.—The post-mortem examination of the heart was made by Dr. Larkin.[2] "The heart showed a calcareous nodule, situated precisely in the path of the bundle of His. This nodule was evidently the cause of the complete heart-block observed during life."

* Submitted for publication Dec. 5, 1913.

* From the Pathological Laboratory of Columbia University at the College of Physicians and Surgeons, New York City, read in abstract before the New York Pathological Society, Nov. 12, 1913.

† A. F. Stanley Kent has described in detail another connection between auricle and ventricle beside the bundle of His situated at the right lateral border of the human heart. (Proc. Roy. Soc. London, 1914, Series B, Vol. No. B594, Biological Sciences, p. 198.)

1. Bishop: Am. Jour. Med. Sc., 1910, cxxxix, 62.

2. Bishop and Larkin: Proc. New York Path. Soc., 1909, New Series, IX, 73.

The heart was received from Dr Bishop in formaldehyd solution and the measurements of the preserved heart revealed nothing of interest

The edges of both mitral cusps were shrunken and deformed and one could feel a hard calcareous deposit all along the attachment of the posterior cusp The aortic cusp of the mitral valve was thickened throughout and contained irregular, rough, hard calcareous deposits This calcareous mass was continuous with that described above in the upper portion of the interventricular septum and in the space between the anterior and right posterior cusps of the aortic valve, that is, directly in the normal pathway of the bundle of His The cusps of the aortic valve were markedly thickened The wall of the aorta itself showed patches of atheroma The coronary arteries, both right and left, presented a very marked sclerosis with patches of atheroma

Microscopic Examination.—Such a portion of the heart was removed for microscopic examination as would include the calcareous mass which seemed macroscopically to be situated precisely in the path of the bundle of His, namely, such a portion of the septum between the two halves of the heart as included the node, stem, and the branching of the auriculoventricular system The upper margin of this block ran along the upper edges of the aortic leaflets, and the lower margin parallel with this 3 cm below the lowest part of the pars membranacea septi

This piece of tissue was decalcified with nitric acid, embedded in paraffin, cut from above down, parallel with the upper margin, in sections 15 to 19 microns thick Altogether 2520 sections were cut and for a preliminary study every fortieth mounted Finally about every fourth section from Section 1322 (where the node was first seen) to 1618 (well beyond the break in the continuity of the auriculoventricular system), and about every tenth from 1618 to 2440, was mounted All the sections were stained with Weigert's iron hematoxylin and van Gieson's picric acid fuchsin solution except Sections 1392, which was stained with hematoxylin and eosin, and 1334, which was stained by Roehl's method for calcium

The auriculonodal connections are attenuated (especially the strand to the left) but are uninterrupted A very few round cells and still fewer polymorphonuclear cells are found at this junction and in the node itself

The node lies, as usual, to the right of the central fibrous body, but is peculiar in that it is largely cut off from the auricular musculature by fatty tissue It consists of a network of pale, fine muscle fibers with crowded nuclei The nodal artery shows increase of connective tissue in the intima and media, indeed there is sclerosis of the vessels throughout the whole of all the sections

The node and stem are continuous The stem is characterized by a greater vascularity, a more marked cellular infiltration and by the presence of more connective tissue than the node, which characters are so marked in the auriculoventricular system at and near the bifurcation of the stem as undoubtedly to be beyond normal limits The stem progresses normally through the membranous septum in a short course and comes to lie under the endocardium of the left ventricle, when abruptly at its forward end it abuts on a calcified necrotic sphere there bifurcating to form what were judged to be stumps of the right and left branches The calcified sphere, which lies directly across the path of the stem at its bifurcation, stretches from the endocardium of the left ventricle almost to that of the right

This calcified sphere forms part of an area of calcification which begins just below the anterior aortic (coronary) cusp and involves the stump of the mitral valve, the connective tissue of the auriculoventricular ring and the undefended space, and the surrounding musculature, namely, that of the left auricle and of the basal part of the interventricular septum and of the auriculoventricular bundle The area consists in the main of nodules of calcified necrotic material well encapsulated by connective tissue sheaths In and immediately about some of the encapsulated nodules there is increased vascularity accompanied by pus

cells (mostly round cells but not a few polymorphonuclears) There is also here and there the typical invasion of the calcific nodules by young connective tissue with an occasional giant cell

At one level one of these calcific nodules appears to the right of the ventricular end of the stem abutting on its narrowest part, and comes to lie in an indentation of the stem giving the appearance of compressing it This occurs not far from the bifurcation, namely, at the forward end of the stem, which is composed mostly of thin-walled engorged blood-vessels and connective tissue and proportionately very few muscle-fibers The undoubted increase in connective tissue elements and vascularization which characterizes the ventricular portion of the stem also exists in the stumps of the right and left branches

The stumps just mentioned are, judging by their position probably the true branches and not the result of a mere splitting of the stem owing to pathological conditions The lesion in this angle is, as has already been mentioned, a large calcific necrotic sphere which separates the stem and stumps from the rest of the right and left branches proper (Figure 1) The left branch proper is easily identified, but the identification of the continuation of the right branch we cannot assert with the same conviction

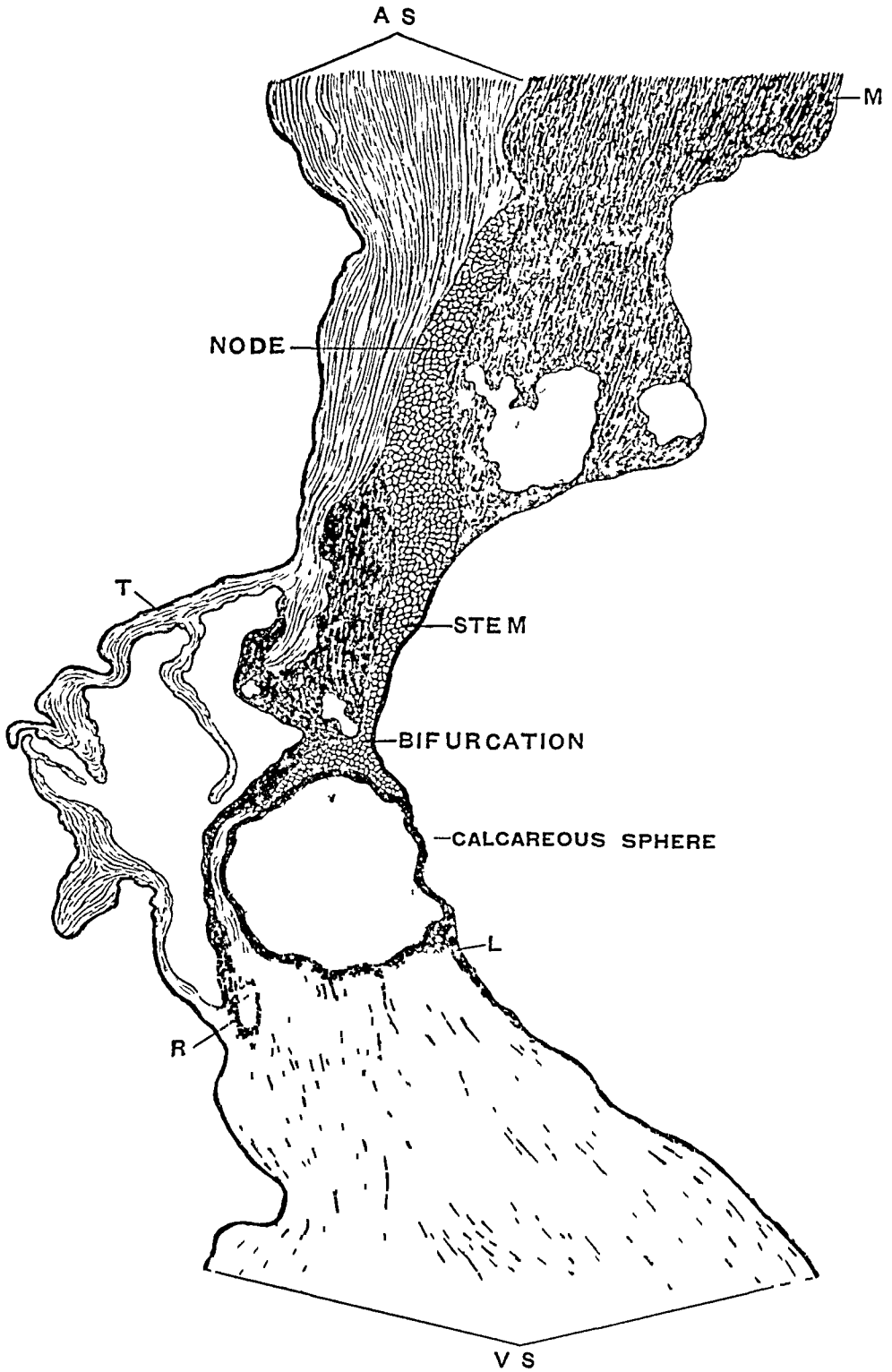
Even on a naked-eye study of the sections it is clear that as one goes from the aortic valve toward the apex of the heart the calcific, necrotic focus involves a more and more extensive area and compromises the auriculoventricular system more and more And microscopic study reveals that in Section 1462 there is no viable muscle-fiber in the stump of the right branch and that in Section 1480 there is not even an indication of the stump of the right branch,[3] that in Section 1502 there are no muscle-fibers in the stump of the left branch, and that there is in the region of Section 1544 from above down a hiatus of about 1 mm (1 026 mm) in the stem where there is no viable musculature At this level the stem region consists of vascularized connective tissue with much cellular infiltration (mostly round cells, an occasional polynuclear and a few cells larger than round cells characterized by more cytoplasm and a less compact nucleus)

To summarize, there are for a short distance (examining from above down) no muscle-fibers in the stem Above this hiatus a large calcific necrotic sphere intervenes between the stem at its bifurcation on the one hand and the right and left branches on the other, and it is only below this hiatus that one branch (namely, the left) is continuous with the body of the stem In brief the right and left branches are cut off from the auriculonodal junction because necrotic tissue is situated in the path of the auriculoventricular bundle

The two branches, cut off as they are from the node, present no abnormalities in their structure

It should be borne in mind that the identification of the "main' part of the right branch as it descends downward is not absolute, since it is nowhere continuous with the stem and was judged by its location, the lack of density of its muscle-fibers and its isolation Farther up, the muscle fibers of the right limb are slightly finer than those of the surrounding musculature, farther down, slightly coarser with occasionally a clear central space At one level a small group of muscle-fibers which lies between the right branch and the epicardium presents great resemblances to the right branch, some of its muscle-fibers have even a clear central space so that this segment of musculature is indistinguish-

3 In spite of the absolute break in the continuity of the right branch, impulses may yet have passed from the stump of the right limb through the ordinary ventricular musculature to the ventricular chamber, for it is not unlikely judging by the anatomical findings in the hearts which we have studied and by physiological considerations that the auriculoventricular system is not completely isolated between its two ends namely all along the line from the auriculonodal connection to the Purkinje fiber ventricular junction



Projection drawing of Sections 1438 and 1442, Case 1—Showing an old calcareous focus, surrounded by connective tissue, which causes complete interruption of the bundle of His At the bifurcation of the stem a calcific nodule (larger in the following sections) seems to press in upon it This indented region of the body of the stem and the stumps of its two limbs present manifestly more thin walled blood vessels and more connective tissue than is normal At a lower level the necrosis encroaches on the stem to such an extent that it is entirely lacking in muscle-fibers for about 1 mm from above down Fibrous tissue is dark, necrotic material is dotted, *A-V* system is honeycombed, *A-S*, auricular septum, *L*, region where isolated left branch of *A-V* system appears in sections lower down, *M*, mitral valve, *R*, isolated right branch, *T*, tricuspid valve, *V-S*, ventricular septum

able from the right branch as to its structure Now and again the right limb presents more connective tissue than is found in the neighboring myocardium

The left branch is characterized by somewhat more connective tissue and is on the whole somewhat paler staining than the surrounding musculature, with its individual muscle-fibers slightly coarser and not so densely constructed, though only rarely presenting a clear central space

The vessels of both the specialized and typical musculature present sclerosis of the intimal and mesial coats The muscles about the vessels (especially perhaps those of the outer coat of longitudinal fibers)[4] are finer and paler than the cross-striated typical cardiac muscle-fibers and resemble those of the nodal musculature of the heart The capillaries are everywhere engorged and in places small extravasations have occurred into the tissues, possibly signifying nothing more than ante-mortem or post-mortem stasis

There is a good deal of connective tissue in the interventricular septum but no evidence of recent inflammation Besides the calcified area in the upper part of the interventricular septum (in the angle of bifurcation of the auriculoventricular system and crowding in on the stem) there are isolated areas with lime deposit in a region of much connective tissue between the right and left sides of the interventricular septum and in some connective tissue of the right ventricular musculature

The smooth muscle of the left endocardium shows more connective tissue than normally As usual, its muscle fibers, like those about the vessels, resemble the muscle-fibers of the nodes of the heart

The endocardium is normal, except that part of the left endocardium which covers the degenerated area where the cellular infiltration extends into it and dense connective tissue replaces the usual loose subendothelial tissue to quite an extent

The epicardium of the posterior surface (the only epicardium on the sections) presents a thick, compact connective tissue layer under a not inconsiderable part of the epithelium In this layer there is (below the level of the node and stem) both a scattered and a dense cellular infiltration, that is, round cells, a few cells with larger cell body and larger vesicular nucleus, and an occasional polynuclear are found scattered and in masses A most imposing focus of dense cellular infiltration is seen in the subepicardial fatty tissue immediately under the compact connective tissue layer in the lowest sections

SUMMARY

Though the auriculonodal junction is not very powerful, it is not, apparently, inadequate, but there is an actual interruption of the continuity of the auriculoventricular structure along its course to account for the symptoms in this case, both branches of the conducting apparatus are cut off from the main stem of the auriculoventricular system and from each other There is undoubtedly more connective tissue in the stem and more vascularity (with its attendant cellular infiltration) than is normal, and the upper ends of both limbs almost wholly and the body of the stem for about 1 mm of its length have been demolished by a chronic, necrosing, calcifying process In addition, there is an inconspicuous cellular infiltration at the node, as well as a more

4 This may be due to the direction in which they are cut It should be noted that in this (as well as in other hearts) some of the ordinary cardiac musculature seems paler or more vacuolated than the rest owing to the direction in which the muscle-fibers are cut

marked one of the stem and an imposing accumulation of round cells in the subepicardium, there is sclerosis throughout the coronaries and their intermuscular branches, and an increased amount of connective tissue in the ordinary ventricular musculature and some little calcific necrotic tissue

HEART 2

HEART 2.—*Case Report*.—The heart was forwarded to Dr. L. F. Barker of Johns Hopkins Hospital by Dr. D. A. Campbell, professor of medicine, Halifax, Nova Scotia, together with the following notes: "I first saw J. C., a retired mariner, in the summer of 1905, shortly after he was admitted to an institution for the care of the aged. He was then in his ninety-fifth year. His bodily health was excellent. He was quite corpulent, but unusually active. He was generally cheery and bright in disposition. His mental faculties were well preserved. Physical examinations disclosed the existence of some enlargement of the heart, and the presence of a very loud, harsh, aortic systolic murmur. The pulse rate was 60 and quite regular. During the following winter he had a sharp attack of influenza, from which he recovered completely. During the summer of 1906 he was ill the greater part of the time, but never wholly confined to bed. The most pronounced symptoms were mental depression, cyanosis, bradycardia (the pulse rate varying from 10 to 40 without any irregularity), slight seizures resembling petit mal occurring very frequently some days, and Cheyne-Stokes breathing especially at night. On account of the patient's being so fat, no superficial veins could be noticed in the neck. Various remedies were tried to afford relief without much success. Finally belladonna was given in moderate doses and improvement followed. That belladonna favorably influenced the conditions was evident from the fact that when the remedy was discontinued for ten days or so most of the symptoms returned.

"He continued to keep fairly well for a period of ten months, using the belladonna the greater part of the time.

"Early in August, 1907, the symptoms returned and gradually increased in severity, causing his death a month later. The bradycardia was not so marked as before, 30 being the lowest pulse rate noted, and the epileptiform seizures were infrequent. The more prominent terminal symptoms were marked cyanosis, Cheyne-Stokes breathing and *total disappearance of the radial pulse and cardiac impulse for periods varying from thirty to sixty seconds in duration*. This very unusual modification of the pulse was noted for five days before death, and seemed to be closely connected with Cheyne-Stokes respiration. The pulse usually, but not always, disappeared during the period of dyspnea, returning during the period of apnea. No sound whatever could be heard over the cardiac area when the radial pulsations were absent."

Macroscopic Examination of the Heart.—The heart was received from Dr. MacCallum in formaldehyd solution. Grossly the fixed heart presented no abnormality in the region of the conducting system which would account for heart-block.

Microscopic Examination of the Heart.—For microscopic examination the following blocks of tissue were excised.

1. The region of the sinu-auricular node. This block consisted of the auricular wall on either side of the sulcus terminalis of His extending posteriad as far as the interauricular septum and including a part of the superior and inferior venae cavae, and extending anteriad so as to include some of the pectinate musculature (a strip of about 0.5 cm.). Moreover, this block extended along both sides of the crest of the right auricle, thus including not only the part already described on the lateral aspect of the auricle on either side of the sulcus, but also the auricular wall on the median aspect facing the aorta. On the lateral aspect the area reached almost to the auriculoventricular groove, on the median face it

extended from the crest down for about 1 cm toward the insertion of the aorta and was a continuation, as regards its width, of the lateral portion

2 The auriculoventricular septum, containing the auriculoventricular node, the main stem, and the beginning of the branches The sections were cut horizontally, approximately parallel to the stem

3 A portion of the upper ventricular septum (left side) containing a part of the left branch of the bundle of His

The first two pieces were embedded in celloidin-paraffin and cut in sections 15 microns thick Every fifth section was mounted and stained with Weigert's iron-hematoxylin and van Gieson's picric acid fuchsin solution A few sections were stained with orcein The third piece was frozen, cut, and one section stained with Scharlach R, one section stained with iron-hematoxylin and van Gieson's solution, and finally one with orcein

Sinu-Auricular Node Region—The sinu-auricular node is sclerotic There are small compact masses of connective tissue between the muscle fibers, there is more connective tissue at the cephalic than at the caudal end of the node, where the muscle-fibers are separated by looser connective tissue instead of by "wads" Even allowing for individual variation within normal limits, the increase of connective tissue in this case is so marked throughout the entire extent of the node that it may be safely stated to be abnormal No such fibrosis is present in the musculature immediately about the node or in the other portions of the right auricle examined In the main the nodal fibers themselves may be described as lacking in cross striations There is, however, the typical gradual transition from nodal into ordinary auricular musculature The nodal artery, present in this heart, shows sclerosis of the media and a slight endarteritis Some of the smaller branches too show these changes as do also the artery on its way to the node and some of the vessels among the ordinary cardiac musculature, occasionally a vessel presents obliterating arteritis Some of the sections of the arteries have an outer layer of longitudinal smooth muscle-fibers arranged in small bundles[5] Some of the nuclei of the auricular musculature are large and present uneven distribution of the chromatin and sometimes an irregular margin[6]

Here and there in the pectinate and other ordinary cardiac musculature as well as in the specialized musculature of the Keith-Flack node, a very few cells which resemble lymphocytes are seen They are slightly larger, have more cytoplasm, and their nuclei are more vesicular, less compact than those of lymphocytes At one place in the nodal fat there is a small group of these cells Larger collections of them appear in the epicardium and subepicardium, the largest at the level of the pace making apparatus Sometimes they are found about the subepicardial nervous elements in the neighborhood of the node Now and again a nerve passes from the subepicardium into the sinu auricular system Once a ganglion cell was noticed alongside a capillary in the very center of the nodal tissue

In general the effects of the pathological changes in the node of Keith and Flack are not yet established, and we do not venture on judging the significance of the sclerosis of this structure in this case

Region of the Auriculoventricular System—The connections between the auricular musculature and the node of Tawara are scanty The node in the sections is an irregular semicircle, the diameter of which lies along the central fibrous body, and the circumference of which is almost entirely surrounded by fat tissue, thus practically separating the nodal from the auricular musculature There is an abnormal amount of fat tissue in the region of the interauricular septum The node contains very little connective tissue

5 Such bundles of longitudinal smooth muscle fibers are normally found in all hearts

6 The nuclei of the swollen type mentioned above were described by Dr A E Cohn in *Heart*, 1912, iv, 24

Cross sections of the artery in the specialized musculature present sclerosis of the intima and the media Some of the very small vessels have a marked diminution of their lumina, but it is impossible to say which of the two inner coats is involved There is intimal and mesial sclerosis of the arteries in the interauricular and interventricular septa, and of the coronary artery (with atheroma) There is calcific deposit in the wall of the aorta not only near the opening of the right coronary but even nearer to the auriculoventricular system than that[7]

As was to be expected in sections of a heart from a patient advanced in years, lipochrome granules are seen in the muscles of the auricle, ventricle, and auriculoventricular system Some of the nuclei of both the auricular and the ventricular muscles were of the "swollen" type already described The smooth muscle of the left endocardium presents increase of connective tissue as does the ordinary interventricular musculature especially on the left side

Though the stem is long and slender, there is nowhere a break in the continuity of the auriculoventricular system

Upper Ventricular Septum—The piece from the upper part of the septum on the left side of the heart presents an increased amount of connective tissue in the ordinary cardiac musculature but no evidence of recent inflammation There is also a slight increase in the connective tissue surrounding some of the vessels, all three vessel coats are sclerotic There is no fatty infiltration of any of the musculature The endocardium appears to be normal

SUMMARY

While it cannot be affirmed that the slightness of the auriculonodal junction is the causative factor in this case of Adams-Stokes disease, yet it is of importance in the light of the fact that Armstrong and Monckeberg[8] reported an exquisite case of lymphangio-endothelioma of the auriculoventricular node in which it was judged that the break between auricular and nodal musculature was the cause of the heart-block

The condition of the blood-vessels in the aged patient may have been an accessory factor

We should expect to find some cellular infiltration in arteriosclerotic hearts, and we do not know that there is any relation between the increase of cells in the interstitial tissue of the sinu-auricular node and of some of the ordinary auricular musculature and the clinical symptoms in this case

It should be recalled that the interventricular septum, especially on the left side of the heart, presents an increased amount of connective tissue, and that some of the nuclei were of the "swollen" type with scattered chromatin and sometimes with irregular margin

In brief, the most obvious explanation of the symptoms in this case is the auriculonodal break

7 In another heart (from a patient without data as to heart block) though calcareous nodules were found grossly in the neighborhood of the bundle of His, microscopically these did not interfere with the continuity of the auriculoventricular system This heart was kindly given us for examination by Dr W G MacCallum

8 Armstrong and Monckeberg Arch f klin Med, 1911, cii, 144

HEART 3

HEART 3.—*Clinical History*.—The heart was received from Dr W G MacCallum who performed the autopsy and gave us the clinical notes furnished him by Dr M G Porter.

The patient was a white man aged 50. There was no history of syphilis, he had not used alcohol to excess. He had always been strong and healthy until eighteen months before exitus, when he had suffered from several attacks of syncope within a few days. Six months before his death he had a similar seizure and since that time he had frequently complained of giddiness on rising and of shortness of breath. Three days before his death he was seized, after violent exertion, with severe pain in his precordium, radiating down both arms. Subsequently he had repeated attacks of syncope and several epileptiform convulsions with attacks of bradycardia between which the pulse rhythm would be normal. The pulse-rate ranged between 110 and 25 beats per minute. He died in one of his seizures. The urine was acid, specific gravity 1.018, trace of albumin, no sugar. On the day previous to his death a distinct pericardial friction rub was heard over the body of the heart. This was again heard about an hour later ("pericarditis epistenocardia").[9]

Autopsy.—The autopsy revealed the cause of death to be rupture of the wall of the heart and consequent hemopericardium. "The pericardium was found to be filled with blood which was partly clotted. In places the clot was fully 1 cm. thick. There was a ragged hole in the anterior wall of the left ventricle about 1 cm. to the left of the vein that corresponds to the interventricular septum. Some blood clot was adherent to and extended into this ragged opening. The interventricular septum throughout its entire extent was opaque, of a grayish ochre color and was evidently necrotic. This appearance extended to within 1 cm. of the pulmonary orifice where it was sharply marked off from the adjacent normal heart wall. The wall of the left ventricle was greatly hypertrophied, but its tissue was not abnormal.

"The trabeculae were opaque and frequently broken through. In the angle between the wall of the ventricle and the septum was a hole, through which a probe was passed until it extended through the opening on the anterior surface of the heart. Both coronaries were markedly sclerotic. The anterior descending branch of the left coronary artery was completely occluded by a thrombus 3.5 cm. from its orifice. The sequence of events has evidently been coronary sclerosis, thrombosis of the left descending branch, infarction of the interventricular septum, rupture of the heart wall, hemopericardium."[10]

Grossly, then, the heart presented an infarction of the interventricular septum including the neighborhood of the main stem of the bundle of His and its branches.

Microscopic Examination of the Heart.—The heart was received from Dr. MacCallum in formaldehyd solution. For microscopic examination the following blocks of tissue were excised.

1. The region of the sinu-auricular node as described under Heart 2.

2. The auriculoventricular septum containing the auriculoventricular node, the main stem and the beginning of the branches.

3. The ventricular septum adjacent to Block 2 (just below it) with the posterior papillary muscle of the left side and the underlying ventricular wall and a piece of the apex.

4. A portion of the upper ventricular septum (*left side*) *containing a part* of the left branch of the bundle of His.

5. The anterior papillary muscle of the right ventricle with chordae tendineae.

9 Sternberg. Compt. rend. XVI Cong. internat. méd., Budapest, 1909, Section VI, Méd. Interne, p. 396; Verhandl. d. XXIX Deutsch. Cong. f. inn. Med., 1912, p. 444.

10 MacCallum. Johns Hopkins Hosp. Bull., February, 1908, No. 203, xix, 50.

6 The base of the anterior papillary muscle of the left ventricle and the underlying ventricular wall

7 A portion of the wall of the left auricle

Blocks 1, 2 and 4 were embedded, cut, mounted and stained like the corresponding pieces in Heart 2, piece 3 like blocks 1 and 2 above except that only every fortieth section was stained The remaining blocks of tissue (5, 6 and 7) were embedded in celloidin-paraffin, cut 15 microns thick and two sections of each stained

Left Auricle (Block 7) —To begin with the least important block of tissue The epicardium and adjoining fat and muscle of the wall of the left auricle present cellular infiltration (only an occasional polymorphonuclear leukocyte here) The infiltration is most marked in the epicardium and subepicardium, one small focus was seen in the endocardium There is sclerosis of the media and intima of the vessels

Region of the Sinu-Auricular Node (Block 1) —Unlike the portions from the interventricular septum, the block of tissue in the region of the sinu auricular node presents a comparatively slight cellular infiltration The round cells (only a few polynuclears are to be seen) are present in the epicardium and subepicardium, to a less extent in the nodal tissue, and to still less an extent in the ordinary myocardium There is practically no invasion of the endocardium The area near the inferior vena cava is somewhat more involved than the rest of the ordinary cardiac musculature The most marked infiltration in this block is found in two regions, namely (1) in the epicardium at the level of the node and (2) about the subepicardial nervous elements and the musculature on the median face of the crest near the interauricular septum (i e, the region of the synapse of the vagus as described by Marchand and Meyer[11]) There is a negligible increase in the amount of connective tissue in the head of the pacemaking apparatus The nodal muscle fibers are much coarser than those of Heart 2 and show cross striations clearly There is the usual gradual transition into ordinary cardiac musculature There are a few pus cells in the media of the nodal artery The vessels, both of the specialized and typical musculature, present sclerosis of the two inner coats As usual, some of the sections of the arteries present an outer layer consisting of bundles of longitudinal non striated muscles Some of the nuclei of the musculature of this piece of tissue are of the swollen type At one point the node encloses ordinary musculature Here and there a nerve fiber from the subepicardium is seen to enter the nodal structure, and in its very center a ganglion cell was observed

It does not seem possible that the very slight cellular infiltration of the pacemaking system in this case could account for any decided influence on the functions of the heart

Region of the Upper Auriculoventricular System (Block 2) —A very few infiltrating cells — for the most part lymphocytic, rarely polymorphonuclear — are seen in the conducting system The right branch, and the stem (at the level of its division into two branches) and the central fibrous body near by present more pus cells than the node, the upper stem, and the left branch Close at hand, at the basal end of the ventricular septum is an infected infarct with pus cells in the capillaries and adjoining tissues There are pus cells elsewhere (everywhere round cells exceed the polynuclears in numbers), namely in the epicardium, subepicardial fat, auricular musculature, endocardium, tricuspid and mitral and aortic valves But the most pronounced lesion in this block is situated in the typical ventricular musculature *near* the junctional tissue Here the musculature has taken an unusual brown tint and many of the muscle nuclei seem to have disappeared Indeed, some of the muscle fibers themselves have apparently disappeared in their entirety and have been replaced by connective tissue The

11 Marchand and Meyer Arch f d ges Physiol, (Pflueger's), 1912, cxlv, 401

remaining muscle-fibers are separated by columns and masses of pus cells and are shrunken The central fibrous body is a little larger than normal Practically all the vessels present intimal and medial sclerosis, such are the nodal artery, the aorta, the coronary artery, and, in brief, other vessels in the auricular and ventricular septa Some of the nuclei of this block of tissue are of the swollen type The smooth muscle of the left endocardium is normal

Region of the Lower Auriculoventricular System and of the Terminal Arborizations (Blocks 3, 4, 5, 6) —Upper Ventricular Septum In Block 4, that is, the piece from the upper part of the septum on the left side of the heart, there is histological evidence of the infarction just as there is in the other blocks from the interventricular septum There is a round-celled and polymorphonuclear infiltration of the endocardium, subendocardium, the region of the left branch and especially of the ordinary ventricular musculature where many of the muscle-fibers are separated from one another by pus cells, are shrunken, and, though still showing cross striations, are lacking in nuclei Some of the muscle-fibers have indeed apparently quite disappeared There is fat in both the region of the specialized and ordinary cardiac muscles

Region of the Terminal Arborizations—In Block 3, namely, the ventricular septum immediately below Block 2, no attempt was made to find and trace the right branch of the auriculoventricular system The uppermost part of the posterior limb of the left branch was identified macroscopically as if for dissection and marked with India ink[12] and so found in the sections under the microscope, but as we did not follow the left branch continuously in serial sections from the stem on, we cannot assert that we have identified it In this piece three, there is in the ordinary ventricular musculature, including that of the apex, a continuation of the same lesion (an infected infarct) as described for the other pieces from the ventricular septum, namely, pieces two and four In that portion of the left limb which was marked with India ink there was no cellular infiltration, but ventral (anterior) of this region there is some infiltration of the endocardium and subendocardial musculature where it is not unlikely that the left branch is involved Similarly there is infiltration of the endocardium and subendocardial musculature in the apex On the other hand there is no cellular infiltration of the posterior papillary muscle of the left side Many of the cells under the epicardium and endocardium of the right and left ventricle and of the posterior papillary muscle are broad and pale and vacuolated, some of these may be Purkinje cells, possibly some Purkinje like because of the direction in which they are cut and others perhaps because of a Purkinje cell variety of muscle atrophy[13] Some of the nuclei of the cells are of the swollen type with unevenly distributed chromatin and irregular margins The coats of the blood-vessels present an increased amount of connective tissue

The anterior papillary muscle and chordae tendineae of the right side (Block 5) are normal except for some nuclei of the swollen type, the base of the anterior papillary muscle of the left side and underlying ventricular wall (Block 6) present pus cells in the epicardium and subepicardium and sclerosis of the two inner coats of the vessels

In short, an almost negligible number of lymphocytic and a still lesser number of polymorphonuclear cells have invaded the auriculoventricular system, and that is all, therefore from an anatomical standpoint the conducting mechanism is in a condition to functionate, but from a physiological viewpoint the sclerosis of its vessels and the presence of even but a few pus cells in the junctional tissue

12 According to Nagayo [in Beiter z path Anat u z allg Path (Ziegler's) 1909, xlv, 295] the stripes seen macroscopically are due not only to the presence of the branches of the conducting system, but also to bundles of smooth muscle fibers

13 Cohn and Lewis Heart, 1912, iv, 24

and finally the neighboring infected infarct suggest an inefficiency of circulation and an abnormal condition sufficient to account for heart block

DISCUSSION

The sclerosis of the coronary arteries and its branches in these three aged patients suggests that an inadequate circulation played a part in the production of the symptoms of Adams-Stokes syndrome in all three

As was expected in arteriosclerotic hearts, some increase of the cells in the interstitial tissue was observed, but we do not know what may have been its significance in these cases

If these are three cases of heart-block, as is suggested by the Adams-Stokes syndrome, then they form a series, Case 1 with an absolute break between the stem at its bifurcation and the two limbs right and left. Case 2 with its inadequate auriculonodal junction and Case 3 without a break in the continuity of the auriculoventricular system

In the first case described, a lesion to account for heart-block was seen, there was complete transverse destruction of the tissue of the bundle of His In the second case, it is impossible to state to what extent the sclerosis of the sinu-auricular node and the fact that the auriculonodal junction is slight, affected the functions of the heart And in the other case, though histological investigation brought to light no such lesion of the auriculoventricular system (i e, partial or complete break in its continuity), as has been frequently regarded the ultimate anatomical basis of the affection, yet the findings are indicative of such a disturbed circulation and abnormal condition in the neighborhood of the conducting apparatus as might profoundly influence its functional capacity

SUMMARY

1 The hearts of three patients with Adams-Stokes syndrome presented, the first a calcific mass in the region of the auriculoventricular bundle, the second no gross lesion across the conducting system to account for the symptoms, the third infarction of the ventricular septum

2 The first patient is reported to have denied a syphilitic history, in the second case the past history is not given, and the third patient is said to have had no lues All three were well along in years with some sclerosis of the vessels and some increase of cells in the interstitial tissues

3 The most noteworthy lesions in each case were in the first, an absolute break in the course of the stem of the auriculoventricular system, in the second, the slight auriculonodal junction and a sclerosis of the sinu-auricular node, in the third, an infected infarct of the ordinary ventricular musculature near the junctional tissue

4 The possibility of insufficient circulation and of a lesion in the ordinary ventricular musculature as agents in the production of heart-block, are suggested

We are greatly indebted to Dr W G MacCallum and Dr L F Bishop for the privilege of describing these cases and to Dr A E Cohn for his valuable suggestions

THE INTRAMENINGEAL TREATMENT OF TABES AND CEREBROSPINAL SYPHILIS[*]

THOMAS R BOGGS, M D, AND R R SNOWDEN, M D
BALTIMORE

The treatment of syphilis of the central nervous system has always been unsatisfactory The cerebrospinal system is a closed one, and the diffusion of drugs from the circulating blood into the cerebrospinal fluid is slow and incomplete For this reason organisms which have invaded the nervous tissue or meninges remain more or less protected from the drugs which may be present in the vascular system For a number of years the desirability of the direct application of antisyphilitic drugs has been recognized Horsley[1] went so far as to advocate the "heroic" treatment of opening into the subdural space and washing out with mercuric chlorid (1 1,000) solution, but this procedure proved too irritating

With the advent of salvarsan and neosalvarsan, hope was entertained that this therapeutic agent could be injected intraspinally, but investigation showed that it also damaged the tissues Wechselmann[2] found that small amounts (1 mg) when injected into the brains of dogs and rabbits at a concentration of 1 500 and 1 1,000 produced convulsions, weakness of the limbs and death in from two to fourteen days, but small doses, from 0 5 to 0 25 mg, in greater dilution (from 1 2,000 to 1 4,000) caused no reaction Later he reported[3] the intraspinous injection of neosalvarsan in four patients without reaction, giving each from 0 5 mg to 3 mg Marinesco[4] reports thirteen cases in which neosalvarsan was given intraspinally, the dose averaging about 5 mg In two patients no reaction occurred, but in the remainder the reaction was severe, thus, eight patients developed incontinence of urine or retention, three showed weakness of the extremities and in one case anesthesia of the rectum, legs and buttocks occurred Ellis and Swift[5] have carefully studied the effects of the intraspinous injection of salvarsan and neosalvarsan in monkeys They injected small quantities and considered the cell-count as an indication of reaction due to irritation

* Submitted for publication Dec 30, 1913

* From the Medical Clinic of the City Hospital at Bay View

1 Horsley, V Neurol Centralbl, 1910, xxiv, 257

2 Wechselmann, W Die Behandlung des Syphilis mit Dioxydiamidoarsenobenzol, "Ehrlich Hata 606," Berlin, 1911, p 11

3 Wechselmann, W Deutsch med Wchnschr, 1912, xxxviii, 1446

4 Marinesco Diatet u physik Therap, 1913, xvii, 194

5 Swift and Ellis Jour Exper Med, 1913, xviii, 428

They found that the intraspinal injection of even minute quantities (0 2 mg) produced profound cellular reactions which persisted over ten days, while 3 2 mg caused, in one monkey, paralysis of the hind legs, which lasted several months The injections of neosalvarsan were, on the whole, less severe, but still gave evidence of irritation in doses as small as 0 045 mg Repeating the injection seemed to increase these effects

The study of the effects of the intravenous or intramuscular injection of salvarsan brought to light a very important fact Plaut[6] showed that the serum of patients who have received salvarsan exerts a definite anti-syphilitic effect, whereas normal serum has no such activity, and the serum of patients treated with iodids and mercury has very little such power Meirowsky and Hartman[7] and Gibbs and Calthrop[8] treated cases of syphilis with good results by injecting subcutaneously serum taken from other patients who had received salvarsan In the same way Henry Alsten[9] successfully treated several cases of frambesia Ellis and Swift[10] took up the study of this serum and showed that, when taken from the patient within six hours after the salvarsan injection, it inhibits the growth of the *Treponema pallidum*, but if taken before treatment, or from six to twenty-four hours after treatment, there is no inhibition They also reported beneficial effects from the intraspinal injection of this serum, with very few irritative phenomena

This spirochetacidal activity of salvarsanized serum was further studied by Gonder,[11] Castelli,[12] and Ellis and Swift,[13] who showed by the use of animals that it had a marked effect on the virulence of several spirochetes, and that this effect was demonstrable only by the injection into animals of the organisms so treated Thus, Swift and Ellis[13] showed that the serum of rabbits treated intravenously with salvarsan, and the serum of patients treated likewise with salvarsan or neosalvarsan exerted an inhibitory effect on the *Spirochaeta duttoni*, and that these spirochetes did not appear objectively (dark field) changed, but they would not cause infection in animals into which they were injected These investigators also found that heating the serum at 56 C (132 8 F) for half an hour markedly increased the activity of the serum, and that this increase is due only in part to the destruction of some inhibiting sub-

6 Plaut Deutsch med Wchnschr, 1910, xxxvi, 2237
7 Meirowsky and Hartman Med Klin, 1910, vi, 1572
8 Gibbs and Calthrop British Med Jour, 1911, i, 809
9 Alsten, Henry British Med Jour, 1911, i, 360
10 Swift and Ellis New York Med Jour, 1912, xcvi, 53 55
11 Gonder, R Ztschr f Immunitatsforsch, Orig, 1912, xv, 57
12 Castelli, G Ztschr f Chemotherap, Orig, 1912, i, 122 and 167
13 Swift and Ellis Jour Exper Med, 1913, xviii, 435

stance They also showed that the cerebrospinal fluid does not possess these inhibitory bodies

The intraspinal injection of serum, taken an hour after the intravenous administration of salvarsan, was first employed by Swift and Ellis as a method of treating cerebrospinal syphilis Their technic[14] was, in brief, the following One hour after the intravenous injection of salvarsan 40 c c of blood are withdrawn directly into a bottle-shaped centrifuge tube, and allowed to coagulate, after which it is centrifugalized The following day 12 c c of this serum are pipetted off and diluted with 18 c c of normal saline solution This 40 per cent solution is then heated at 56 C for half an hour By lumbar puncture the cerebrospinal fluid is withdrawn until the pressure is reduced to 30 mm (water), after which enough of the serum solution is run in by gravity exactly to replace the cerebrospinal fluid withdrawn

During the past year and a half the cases of cerebrospinal syphilis in the City Hospital at Bay View have been treated along the lines laid down by Ellis and Swift, with one important change—we injected undiluted serum in large quantities (25-35 c c) Thus, there was given, in many instances, twice as much actual serum at a single injection as was given by Ellis and Swift The technic was the following Salvarsan, 0 6 gm , was given intravenously, having been made up in water distilled within an hour of time of using, and the solution was injected at once One hour later from 75 to 100 c c of blood were withdrawn into a large, sterile tube, and this was set aside to clot After from one to eighteen hours the serum was pipetted off and centrifugalized at high speed for fifteen minutes in order to throw down any red blood-corpuscles present The serum was then heated in a water-bath at 56 C for half an hour and the intraspinal injection made at once A lumbar puncture was done and the spinal fluid was slowly and carefully drained off into a graduated sterile cylinder Not more than 35 c c were removed The serum was then injected, a syringe and two-way stop-cock being used, the serum being drawn back into the syringe from the flask and then slowly injected through the needle Great care was taken not to use any undue pressure in running in the serum Enough was injected to replace exactly the amount of spinal fluid removed, whether this was only a few cubic centimeters or as much as 35 c c After the injection all pillows were removed, the foot of the bed elevated and the patient kept on his back in this position for at least an hour The treatments were repeated at intervals of seven to twenty days, when possible, depending in part on the way in which the patient reacted

The brief protocols of eight unselected cases are appended as illustrative of the method, with note of some of the difficulties and dangers,

14 Swift and Ellis THE ARCHIVES INT MED , 1913, xii, 331

and the results achieved. Some cases are still under treatment at the time of this report

REPORT OF CASES

CASE 1.—An unmarried colored laundress aged 37, was admitted suffering from tertiary syphilis with cerebrospinal infection and syphilitic cerebral thrombosis

History.—The primary infection occurred twenty years before onset of present illness. No secondaries showed but tertiary lesions appeared during the past five years. The patient had had slight, irregular mercurial treatment during past five years. Severe frontal headaches had existed for a week before admission, with the development of flaccid, left side paralysis, which came on suddenly eighteen hours before admission with no loss of consciousness or convulsions.

Examination.—Complete left side paralysis, which included the muscles of the face, arm and leg, with moderately exaggerated deep reflexes and positive Babinski's sign. Neck stiff and retracted. Several large luetic ulcerations over lower abdomen.

Treatment and Result.—After admission the patient was given three injections of salvarsan and two intraspinal injections. In the first treatment blood was withdrawn seventy-two hours after the injection, and in the second twenty-four hours after injection. When discharged the patient was normal physically except for a slight residual paralysis of the flexors of the left forearm and a left pes equinus. On admission the cerebrospinal fluid showed twenty cells per cubic millimeter, when discharged there were seven cells per cubic millimeter. Wassermann was positive on admission, negative when patient was discharged.

CASE 2.—A married, white window-washer aged 44, was admitted ill with tabes dorsalis.

History.—Primary infection had occurred twenty years before admission. The present illness began five years before admission with sharp, shooting pains in the legs. Extreme ataxia had developed in past two years. The patient had had six weeks' treatment in a German army hospital at the time of primary chancre. About two months later he was given another short course of iodids and mercury.

Examination.—No sphincter disturbances. Argyll Robertson pupils present, knee-jerks absent, muscle sense in legs much impaired, marked ataxia, causing great difficulty in walking.

Treatment and Result.—Two intravenous injections of salvarsan and two intraspinal injections were given with consequent improvement. On admission the patient was very ataxic and suffering severely with lightning pains. After the second treatment the lightning pains had ceased entirely and he could walk much better. He felt so much improved that he refused to remain for further treatments.

CASE 3.—An unmarried white man, a clerk, aged 37, on admission was found to be afflicted with tabes dorsalis.

History.—The primary infection had occurred nine years prior to admission and seven years before onset of present illness, which was accompanied with lightning pains, gradually increasing unsteadiness in walking and dimness of vision. Bladder symptoms developed about a year after onset. At the time of the primary chancre the patient had local treatment and took pills by mouth for one month, during the past eighteen months, mercury and iodids continuously. One year ago he was given an intramuscular injection of salvarsan and a second three months later.

Examination.—Argyll Robertson pupils. General enlargement of lymph-nodes. Knee jerks absent, muscle sense completely absent in the legs, moderate dulling of tactile and pain sense. Romberg sign markedly positive. Positive Wassermann in blood-serum and spinal fluid and ten cells per cubic millimeter in spinal fluid.

Treatment and Result—Four intravenous injections of salvarsan, three of neosalvarsan, and six intraspinal injections were given Total salvarsan 2 7 gm, serum 116 c c, time, seventy nine days On admission the patient was unable to walk on account of the ataxia At present he walks fairly well without assistance He is now free from the lightning pains The cells in the spinal fluid dropped from ten to six per cubic millimeter, and Wassermann reaction has changed from complete fixation to a very faint fixation The reactions were very severe, prostrating the patient, and for this reason the treatments were discontinued

CASE 4—An unmarried, white salesman, aged 37, was admitted with cerebrospinal syphilis and syphilitic lateral sclerosis

History—The primary infection was incurred fifteen years before onset of present illness two and a half years ago, when unsteadiness in walking developed, soon followed by weakness and stiffness of the legs Paresthesias, numbness, girdle sensations and bladder symptoms developed within eighteen months The patient has had several short mercurial courses by mouth and by inunction

Examination—General enlargement of lymph-nodes Pupils unequal but react normally Knee jerks greatly exaggerated on both sides, ankle clonus present and Babinski sign positive Legs stiff and spastic and held in adduction Patient walks with difficulty Wassermann on blood serum, 60 per cent fixation Positive Wassermann and twenty-five cells per cubic millimeter in cerebrospinal fluid

Treatment and Result—Two intravenous injections of neosalvarsan six of salvarsan and eight intraspinal injections were given, total salvarsan, 3 9 gm, serum 178 c c, time, 195 days (two admissions) On admission the patient was so spastic as to be unable to stand on his feet and he was suffering severely with girdle sensations These disappeared immediately and the spasticity became distinctly less marked until he can now walk without assistance and can go up and down stairs In the spinal fluid the cells have fallen from twenty five to five per cubic millimeter, and the Wassermann from complete fixation to very faint fixation

CASE 5—A married white man, blacksmith, aged 45, was admitted with tabes dorsalis, partial optic atrophy and tertiary syphilis

History—Primary infection appeared as a chancre twenty-two years before admission Present illness began four years before admission with dimness of vision, soon followed by lightning pains which have gradually become more and more intense Unsteadiness in gait has developed during the past two years, no sphincter disturbances At the time the chancre appeared a few local applications of calomel powder were made, no medicine was given internally During the past four years the patient has had more or less continuous treatment with mercury and iodids

Examination—Moderate enlargement of lymph-nodes Argyll Robertson pupils and primary optic atrophy Knee jerks absent, moderate ataxia of legs Positive Wassermann and thirty-five cells per cubic millimeter in spinal fluid Wassermann in blood serum negative

Treatment and Result—Eight intravenous injections of salvarsan and eight intraspinal injections were given, total salvarsan, 4 gm, serum, 204 c c, time, 108 days This patient had been unable to work for six months on account of the severe lightning pains After the first treatment he was entirely free from pains except for one mild attack The cell count in the spinal fluid fell from thirty-five to five per cubic millimeter, and the Wassermann from positive to negative

CASE 6—A white man, bookkeeper, widower, aged 54, was admitted suffering from tabes dorsalis

History—Primary infection occurred twenty-four years prior to admission Initial lesion was not known, possibly a chancre Two years before admission

the patient had an acute retention of urine, and examination at that time showed inactive pupils, knee-jerks faint, bladder atonic and anesthetic, and a positive Wassermann in spinal fluid Eighteen months ago he began to suffer with severe pains in the back, coming on in paroxysms about every half hour, worse at night A hemiplegia, three years ago has cleared up almost completely, the right side having been involved The patient has had several short courses of iodids and mercury by mouth during the past two years

Examination—Argyll Robertson pupils Unilateral sweating involving the whole left side of the head and neck Slight residual weakness and exaggeration of reflexes of right arm and leg Knee jerk almost absent on left Wassermann in blood serum negative Positive Wassermann and sixty-two cells per cubic millimeter in spinal fluid

Treatment and Result—Two intravenous injections of neosalvarsan, seven of salvarsan and nine intraspinal injections were given Total 3 9 gm, serum, 186 c c, time seventy-seven days When admitted the patient was almost prostrated on account of the severe and constant pain in the back This steadily became less intense and the patient was quite comfortable when discharged The cells in the spinal fluid fell from sixty-two to four per cubic millimeter and the Wassermann changed from positive to negative

CASE 7—An unmarried colored man, a cook, aged 45, was admitted under diagnosis of tabes dorsalis with optic atrophy

History—The primary infection had occurred twenty-seven years before onset of present illness, which began five years before admission, with unsteadiness and weakness of the legs, the subsequent history is typical There has been incontinence of urine for three years Lightning pains have been constant but not severe Numerous gastric and laryngeal crises have occurred during the past year The patient has had failing vision for eighteen months For the past year the ataxia has been so severe that he has been practically helpless The patient had a short course of local treatment at time of chancre, mercury and iodids by mouth during past year

Examination—Patient greatly emaciated Complete loss of muscle sense in the arms and legs with exaggerated ataxia of the muscles of the face, arms and legs Argyll Robertson pupils Deep reflexes of arms and legs absent Wassermann in blood serum negative Positive Wassermann and thirty-six cells per cubic millimeter in spinal fluid

Treatment and Result—Three intravenous injections of salvarsan and two intraspinal injections were given Total salvarsan, 1 2 gm, serum, 35 c c, time, thirty-six days Patient suffered an extreme reaction after the second intraspinal injection which was characterized by severe respiratory depression Immediately after the injection, at 4 p m, it was noticed that the patient seemed slightly dazed and did not answer questions so quickly as usual, although he was perfectly rational Twenty minutes later the mouth began to twitch and pull to the right, the eyes turned to the right, and the right arm and leg began to jerk slightly The respirations suddenly dropped to four a minute, and were very irregular, the sequence usually being two or three spasmodic respirations followed by a long period of apnea, sometimes lasting three quarters of a minute The jerking and twitching soon ceased and the patient became quite flaccid At 6 p m it became necessary to give artificial respiration for about half an hour A lumbar puncture was done at 7 p m, and 10 c c of the spinal fluid, tinged with the serum, were allowed to drop out At 8 p m another puncture was done and the spinal canal washed out three times with normal saline It was noticed that the intraspinal pressure varied with the respirations in the normal manner, and there was no noticeable change in the condition of the patient when the canal was drained or filled with 35 c c of normal saline The condition of the patient remained about the same until 1 a m, when he suddenly sat up in bed, recognized those about him and talked rationally In about fifteen min

utes he again suddenly sank into apparent coma, with spasmodic and irregular respirations, four or five to the minute, and remained thus until 4 a. m., when, as before, he suddenly recovered, was perfectly rational and showed no signs of weakness. When questioned the patient asserted he was entirely conscious at all times and told with a fair degree of accuracy what was done and who was in the room. He suffered no pain, but simply could not breathe although he felt a great desire to do so. The next day he was as well as usual. No more treatments were given and he left the hospital in a week, unimproved.

CASE 8.—An unmarried white man, a painter, aged 47, was admitted suffering from cerebrospinal syphilis.

History.—The primary infection occurred twenty-six years before admission. The patient was admitted on account of acute alcoholism. When examined the eyes showed Argyll Robertson pupils. For the past year he has suffered with pains in the back and giddiness. The patient had a short course of local treatment at time of chancre.

Examination.—Reflexes all normal, knee jerks active. Wassermann on blood-serum negative. Faintly positive Wassermann and fifty-four cells per cubic millimeter in spinal fluid.

Treatment and Result.—Four intravenous injections of salvarsan and four intraspinal injections were given. Total salvarsan, 2.4 gm., serum, 118 c.c., time, thirty-six days. The cells in the spinal fluid dropped from fifty-three to ten per cubic millimeter, the Wassermann remained positive. The patient felt perfectly well and could not be induced to remain longer in the hospital.

From the foregoing cases it is evident that the intraspinal method is a step forward in the treatment of tabes and cerebrospinal syphilis. The patients were uniformly relieved from pain, and as far as locomotion is concerned there was a slow but definite improvement, in some instances amounting almost to restitution to normal. It is in this respect that our cases seemed to do better than those of Swift and Ellis, perhaps on account of the larger doses of serum.

The psychic effect of the treatment is also marked. The patients became encouraged by the quick relief of pain and the first improvements in the control of motion and were faithful in their efforts to use the legs and practice walking, stair climbing, etc. The nutrition also rapidly improved under these stimuli. In fact, this very improvement was a hindrance in our series, for with the class of people under our care it was in most instances impossible to keep them in the hospital long enough to carry the treatment as far as we should have liked to do.

The effect of the treatment on the cerebrospinal fluid was very striking. The cell-count first fell to normal, the Wassermann reaction becoming negative some time after the cells were reduced. It has seemed from our observations that the Noguchi reaction was of little value during the carrying out of this treatment, since such large amounts of globulin were injected into the spinal canal. At any rate, the Noguchi reactions were positive, in most instances, after the cell-count and Wassermann indicated a normal fluid.

Where the patients are intelligent enough to appreciate the necessity for repetition of the treatment until the biologic indices of normal are

maintained, there need be but transient interruptions of regular occupation, lasting forty-eight to seventy-two hours as a rule It is also true that while the risks are minimal, they do exist and in all probability very feeble, advanced tabetics or very acute cases of cerebrospinal lues should not be treated in this way [15]

In those of our cases which were treated with the undiluted serum the reactions were sometimes more severe than those reported by Swift and Ellis—pain in the abdomen, legs and rectum, with occasional nausea and vomiting or dizziness, and in a few instances a little fever We do not feel, however, that these constitute any contra-indication to the larger doses of whole serum, especially in the light of the apparently greater benefit to the patient The reactions produced by the injection of the salvarsanized serum from another patient were very severe, as shown by two of our cases, the donors of the serum receiving an equal quantity of the same lot, at the same time and in the same way, having no reaction at all

It is quite evident that so indirect a method of applying arsenic in the treatment of syphilis of the central nervous system cannot be considered as final, but we believe it is an advance on what has gone before and a spur to the findings of new preparations or new methods more suitable for the accomplishment of the desired results

15 One of us has treated by this method a tabetic with diabetes mellitus The patient was told of the risk, but wanted to take any chance He had had five previous intravenous treatments with salvarsan Eighteen hours after the injection of 20 c c of salvarsanized serum he developed diabetic coma and was very ill for five days, when a large intravenous transfusion of sodium carbonate and glucose turned the scale and he recovered from the coma He was not benefited by the treatment except in the disappearance of the lightning pains

TWENTY-THREE CASES OF PNEUMONIA TREATED WITH ANTIPNEUMOCOCCUS SERUM*

WILLIAM R WILLIAMS, M D
Attending Physician New York Hospital
NEW YORK

Through the cooperation of the Research Laboratory I was able to give a series of patients suffering from lobar pneumonia an antipneumococcus serum, and although the results were somewhat inconclusive, it seems desirable to publish them

The series includes practically all of the pneumonia patients admitted to the service of the Second Medical Division of New York Hospital during the period of the test This division receives all medical patients admitted to the hospital on the even days of the month, while those admitted on the odd days go to the first division Although this method of assignment does not give a strict alternation of patients, yet it seemed justifiable to use the patients on the first division as controls, inasmuch as none of them received any serum

The serum was taken from a horse immunized against many strains of pneumococci [1] Usually it was injected directly into a vein of the arm at a temperature about that of the patient This rule was departed from and the serum was given subcutaneously to two children on account of the difficulty of entering the vein with the needle, and also when second and later doses were given to four adult patients who had received the initial dose intravenously The first doses for adults in the earlier cases was 100 c c This was increased later until, in one instance, an initial dose of 300 c c was given Many of the patients received more than one dose so that the total amount of serum that an individual received ranged from 50 to 750 c c This maximal quantity was given in six doses spread over a period of five days

A complete demonstration of the nature of the infecting organism was not essayed Blood-cultures, however, were made in eleven cases Only one of them (Case 11) showed the pneumococcus, the others being negative In Case 6, pneumococci were cultivated from pus from the pleural cavity Cultures were made from the sputum in several instances [1] The diagnosis was based on the clinical evidences of pneumonia, special weight being given to the presence of rusty sputum and

* Submitted for publication Jan 28, 1914

* From the New York Hospital and Research Laboratory, Department of Health, City of New York

1 See Notes on Serum, Cultures, Etc , following

the physical signs of a definite consolidation of the lung In every case the subsequent course of the illness confirmed the diagnosis of lobar pneumonia and strengthened the probability of the existence of a pneumococcus infection.

The chief points of note in these cases are given in a very condensed form in Table 1, together with the details of the dosage of the serum The time of onset of pneumonia is not uncommonly indefinite Many patients give a history of an indefinite illness lasting for several days, during which time they may not have been confined to bed continuously This may explain the duration of some of the more protracted cases of this series Most of the patients, however, gave symptoms that fixed the beginning of the pneumonia with a reasonable degree of accuracy

A review of the data of Table 1 shows that the series included twenty-three cases with sixteen recoveries and seven deaths, a mortality of 30 5 per cent Of the seven patients who died, three were alcoholics, one of whom had suffered from two previous attacks of pneumonia, and all were very actively delirious A fourth death was that of a baby (Case 4), who, in addition to pneumonia, had multiple abscesses of the scalp, double purulent otitis media, and empyema followed by pneumothorax The remaining three deaths seemed to have been due to the frank intensity of the pneumonia

Of the sixteen patients who recovered, eight had defervescence by crisis and eight by lysis The crisis occurred on the following days fourth, fifth to sixth, sixth, seventh, ninth, eleventh, twelfth, and on the eighteenth to nineteenth The onset of the lysis in the other cases in which the patients recovered occurred on the following days sixth, seventh (three cases), eighth, tenth and on the thirteenth day In the remaining cases the onset of the lysis was scarcely definable because the fall of temperature was so gradual Five patients had complications In one instance this was serous pleurisy (Case 5) In cases 6 and 19 empyema developed In Case 4 the signs and symptoms were those of empyema, but an exploratory puncture failed to find the pus On the next day, however, there was evidence of pneumothorax, and on operation considerable pus was found Two days later the child died with edema of the lungs She was extremely ill from the time of admission and had in addition to the pleural complications, double otitis media for which the drum membranes were incised, and also many abscesses of the scalp Otitis was detected in Case 18 also The series offered an opportunity to observe the effects of the administration of large and frequent doses of horse-serum that had not been refined in any manner and that contained no preservative except a trace of chloroform [1] Usually no immediate effects could be detected and the injection of the serum was

TABLE 1 —SUMMARY—

Case No	Sex,* Age in Years, Habits, Etc	Condition of Patient				Blood-Culture, Etc
		Day of Admission	Invasion	Temperature Pulse Respiration	Site Lesion	
1	♂ 12	3	Chill	T 104°–5° P 132 R 28	L L	Sterile
2	♀ 1¼	15 (?)	Chill	T 104 8° P 160 R 50	R U L U	
3	♂ 41 Alcoholic	8	Chill	T 103° P 124 R 48	R M R L	
4	♀ 5	6		T 106° P 140 R 48	R U	
5	♂ 26	2	Chill	T 106° P 116 R 28	L L	Sterile
6	♂ 17	4	Chill	T 105° P 128 R 40	R L	Sterile
7	♂ 30	5	Chilly	T 103 6° P 108 R 32	R U R M	
8	♂ 30	2	Chill	T 105° P 112 R 24	R U	Sterile
9	♀ 29	2	Cough, pain	T 103° P 140 R 40	R L	Sterile
10	♂ 46	7	Chill	T 104 6° P 108 R 32	L L	Sterile

* In this column ♂ denotes male and ♀ female

—OF CASES

Serum Administration			Outcome of Disease			
Day of Disease	Dose, Methods	Effect	Termination of Case	Serum Sickness and Day of Disease it Appeared	Complications	Remarks
4	50 c c intrav	Chill T 3°+	Crisis 4th day Recovery	Purpuric rash, arthritis 8th day		Crisis a few hours after serum
15	30 subc		Crisis 18th and 19th days Recovery			First and second injections without effect
16	30 subc					
17	30 subc					
8	100 intrav.	T 2°+	Resolution			Patient alcoholic, acute delirium, edema of lungs Serum without effect
9	100 intrav		Death 19th day Temp rose 4°, edema of lungs			
10	100 intrav					
11	100 intrav					
6	30 subc	T 2°—	Death	Erythema 19th day	Abscess of scalp, otitis media twenty-first day, pyopneumothorax, 600 c c pus Edema of lungs twenty-fourth day	Serum without any definite effect Patient very ill on admission
7	50 subc	T 3°—				
9	45 subc					
11	50 subc					
14	50 subc					
3	100 intrav		Recovery	Urticaria fever 17th day	Pleurisy with effusion 150 c c aspirated	No definite effect of serum Temperature fell by lysis after last injection
4	100 intrav					
5	100 intrav					
5	100 intrav					
6	150 intrav					
7	200 intrav					
4	100 intrav	T 2°—	Recovery		Empyema (Pneumococcus) operation	Temperature fell gradually after first injection No effect from later injections
7	150 intrav					
8	200 intrav					
10	200 intrav	T 3°+				
6	200 intrav	T 35°—	Recovery	Erythema 16th day		Sharp drop in T, P and R immediately after serum
3	80 intrav	Edema of lungs in one half hour	Crisis 9th day Recovery	26th day, T 104°, 27th arthritis		Patient had symptoms of anaphylaxis Serum stopped, no effect
4	150 intrav		Recovery			Resolution fourth week, no effect from serum
6	175 intrav					
9	200 intrav		Recovery			Temperature fell by quick lysis after serum

TABLE 1—

Case No	Sex,* Age in Years, Habits, Etc	Condition of Patient				Blood-Culture, Etc
		Day of Admission	Invasion	Temperature Pulse Respiration	Site Lesion	
11	♂ 39 Alcoholic	6		T 104° P 100 R 36	L U L L	Pneumo-coccus
12	♂ 16 In extremis	2		T 107°	R L	
13	♀ 2	5		T 105° P 136 R 40	L L	
14	♂ 38 Alcoholic, third attack	6		T 103 6° P 124 R 24	R U	
15	♂ 24	8 (?)		T 105 4° P 102 R 32	R L	Sterile Plasmodium malariae
16	♂ 24	3	Chill	T 105° P 144 R 40	L L	Sterile
17	♂ 4½	11	Chill, cough	T 101° P 118 R 32	L L	
18	♂ 30 Alcoholic	6		T 105° P 128 R 44	L U	
19	♂ 32	4	Chill	T 104 6° P 120 R 44	L L	
20	♂ 13	3	Chill	T 102° P 120 R 48	R L	Sterile
21	♂ 54	6	Chill	T 104 8° P 88 R 24	R U R L	Sterile
22	♂ 20	5	Chill	T 105 6° P 120 R 44	L L	
23	♂ 33 Alcoholic	4		T 102 4° P 100 R 48	R L	

—CONTINUED

Serum Administration			Outcome of Disease			
Day of Disease	Dose, Methods	Effect	Termination of Case	Serum Sickness and Day of Disease it Appeared	Complications	Remarks
6	200 intrav		Death			No effect of serum on T, P and R Died three days later
3	150 intrav		Death			Died in eighteen hours
8	100 intrav		Crisis 12th day Recovery	Erythema 14th day		No effect from serum Crisis fourth day after serum
6	150 intrav	T 2°—	Death		Edema of lungs	Temperature rose steadily, died eleventh day
9 11	250 intrav 50 subc	T 4½°—	Death			Temperature fell after first injection, but rose again and rose after second injection
4 5	250 intrav 50 subc	T 3°—	Death			Temperature dropped after first injection and then rose No effect of serum
12	50 subc	T 2°—	Lysis Recovery			Temperature fell 2° and then to normal after serum
6 9	250 intrav 50 subc		Lysis Recovery	13th day rash and fever, 16th arthritis		Slow lysis interrupted by serum sickness
4 7	250 intrav 50 subc		Lysis Recovery	Arthritis, fever 18th day	Pleurisy with effusion tenth day, 700 cc turbid serum, 12th, 350 cc, 25th pus	Serum without apparent effect
4	250 intrav	General pains	Crisis 5th to 6th day Recovery	Urticaria after two hours		Crisis twenty-four hours after serum
8	300 intrav		Lysis Recovery			Lysis rapid twenty-four hours after serum
6	250 intrav		Crisis 6th to 7th day Recovery			Crisis nine hours after serum
5	200 intrav	Nausea, vomiting, pulse rapid and sweating	Crisis 6th day Recovery			Crisis twenty-four hours after serum

tolerated surprisingly well In five patients, however, symptoms appeared that should be mentioned more in detail

CASE 1 —A boy aged 12, received 50 c c of serum intravenously on the fourth day of his illness A few minutes later the patient had a chill and his temperature rose from 104 to 107 2 F This was followed by a startling drop, and within six hours of the injection his temperature was 97 6 F The fever did not reappear, and convalescence was prompt and complete I am inclined to regard this crisis as independent of the treatment, but at the time the sequence of events was very impressive

CASE 8 —The patient was a negro, aged 30, a rather heavy drinker and a driver of horses When 80 c c of serum had run into the vein, he suddenly developed severe dyspnea, a very rapid and weak heart action, profuse sweating and edema of the lungs The serum was at once stopped Within a few minutes the symptoms began to abate and within half an hour the lungs were nearly clear and his condition was satisfactory The subsequent course of the pneumonia was typical and but moderately severe Six days later, on the ninth day of the disease, defervescence by crisis occurred Convalescence was interrupted only by symptoms of serum sickness

CASE 20 —A boy aged 13, during the introduction of the serum complained of headache, pain in the abdomen and later in the legs The pulse was accelerating and the serum was stopped, but not until he had received 200 c c Two hours later a profuse urticarial rash appeared on the abdomen and lasted a few hours The patient had no late symptoms of serum sickness

CASE 23 —A man, aged 33, a heavy drinker, when 150 c c of serum had been given, became nauseated and vomited These symptoms ceased in about five minutes and 50 c c more of serum were given. At this time the pulse became rapid and the patient broke out in a profuse sweat The serum was stopped and in a few minutes he was comfortable He likewise had no late serum symptoms

The effect of the serum on the leukocytes was determined by frequent blood-counts before and after the injections No constant effects on the white cells could be made out The results of some of these counts are detailed in Table 2

Late symptoms due to the serum were noticed in eight patients In consideration of the large doses of unrefined horse-serum, this is not a surprisingly large number The symptoms were not usually severe

During the time of this experiment the First Medical Division of New York Hospital treated fifteen cases of pneumonia with six deaths, a mortality of 40 per cent, as compared with 30 5 per cent for the serum cases Of their ten cases with recovery, in six, or 60 per cent, there was defervescence by crisis In the serum cases 50 per cent of the patients who recovered had crisis Only two of the control cases had complications, a percentage of 13 3 In the serum cases five patients, 22 per cent, had complicating inflammations It does not seem that these were due to the use of the serum, but the number of patients with inflammations of the pleura (four cases, 17 4 per cent) is uncomfortably large

TABLE 2.—BLOOD COUNTS BEFORE AND AFTER INJECTION

Case No	Dose of Serum	Serum, c c	Leukocyte Counts					
			Before Serum		After Serum			
			No of Cells	Percentage of Polynuclears	Hours After Serum	No of Cells	Percentage of Polynuclears	Note
1	first	60*	25,000	86 5	2	19,000	85	
					4	30,000	87	Crisis
					6	29,000	81	
					18	20,000	69 5	
					27	14,000	44 7	
2	first	30†	15,000	81 0	3	14,500	65	
					5	12,700	70	
					14	10,500	76	
	third	30†	13,000	70 0	2	12,900	70	
					13	11,000	70	
					36	15,900	71	
3	first	100*	50,000	94 5	2	49,500	95	
					4	50,000	91	
					16	55,000	97	
5	first	100*	24,400	85 0	2	26,900	92 5	
					4	25,000	86	
					6	25,400	89	
					8	25,900	88	
					10	24,500	88	
					21	20,000	85	
6	first	100*	27,800	88 5	2	29,000	91 5	
					4	26,900	94	
					6	28,800	93	
10	first	200*	20,800	93 5	2	29,000	94	
					4	27,000	93	
					6	23,000	90	
					18	21,100		
16	first	250*	14,000	86 0	2	16,400	92	
					4	15,400	89	
					6	12,200	84	

* Intravenous injection † Subcutaneous injection

A study of the results of this treatment leaves one doubtful of the influence of the serum, in modifying favorably the course of the pneumonia

The prosecution of this experimental study necessitated much extra work in the wards and laboratory This was performed by the members of my house staff, Drs Howard, Kutil, Alling and Wildman I wish to thank them for their enthusiastic willingness, which made these observations possible

NOTES ON SERUM, CULTURES, ETC

CHARLES KRUMWIEDE, JR, M D, NEW YORK

The serum was prepared by injecting a horse with *pneumococci* from varied sources These cultures were all typical inulin fermenting types They were

isolated from the mouth in cases of Riggs' disease, from the sputum in cases of lobar pneumonia, from empyema, and from the meninges and blood of septic infections following the exanthems The cultures used varied from time to time As fresh cultures were isolated the older cultures were dropped No attempt was made to raise the virulence of these cultures by passage through rabbits or mice Although this may influence the protective value of the serum for these animals, it is open to question what influence such adaptation to a new host has on the curative value in man The cultivation on artificial medium undoubtedly changes the pneumococci, but how far this influences their ability to stimulate curative substances in the horse is unknown The purpose was to prepare an immune serum against the prevailing strains of pneumococci, using the strains as little removed from human sources as possible, and to test the value of such serum for general use, where the immunological tests on each case would be impossible

The injections at first were cultures in broth to which was added horse blood Later the cultures were grown on agar and washed off with water At first the increasing doses were of cultures which had been heated to 55 C for one hour When the dose had reached the maximum decided on, live cultures alternating with autolysate were used The autolysate was prepared by allowing distilled-water suspensions of the cocci to stand in the ice chest for one week The live cultures or the autolysate were added to the heated vaccine, in increasing amounts, until only the unheated culture or autolysate was given

The injections were given once a week subcutaneously The maximum dose equaled the growth on agar in 10 pint Blake bottles The horse was receiving the total amount of live and autolyzed cocci at the time it was supplied to Dr Williams The bleedings were made every two weeks Every effort was made to supply the fresh serum to avoid any possibility of deterioration After the serum was drawn from the clot, several cubic centimeters of chloroform were added to each liter In bottling the serum for use, the supernatant serum was drawn off and the lower portion containing sediment and excess of chloroform discarded

The material from the cases was examined both for diagnosis, and in the hope that the cultures isolated could be studied for their complement-fixing power, with the serum used, and that the protection value of the serum could be tested on the type of pneumococci found This study was not made, partly because of other work, and partly because the strains were found to possess such low virulence for rabbits When the intravenous inoculation of the growth from one blood agar slant was given, only two strains killed, one in forty eight hours, the other after ten days Cultures isolated from the heart's blood of these rabbits did not kill other rabbits when a somewhat smaller dose was used The sputum from Cases 18 to 23, inclusive, was examined They all showed an overwhelming preponderance of typical pneumococci One case of the series showed the presence of an abundance of *Streptococcus mucosus*

BOOK REVIEWS

MATERIA MEDICA, PHARMACOLOGY, THERAPEUTICS AND PRESCRIPTION WRITING FOR STUDENTS AND PRACTITIONERS By Walter A Bastedo, Ph G, M D, Associate in Pharmacology and Therapeutics at Columbia University Cloth Pp 602 Illustrated Philadelphia and London W B Saunders Co, 1913

The author believes that we are at the dawn of a new era of simple and practical therapeutics as the outcome of the adoption of laboratory methods in clinical research For this reason he has given special prominence to the discussion of clinical studies on the action of drugs It is this which gives the book its most striking character, namely, that it is right up to date To the busy practitioner who has not had the time to keep up with the reading of the vast bulk of current literature this book ought to be most welcome To the student this book offers, in a compact and most readable manner, a good practical course in these subjects perhaps more practical and more useful than most of the other books on pharmacology and therapeutics at present in use

It is perhaps somewhat unfortunate that the author has seen fit to use a therapeutic classification, for though it might be admitted that any classification in pharmacology is more or less unsatisfactory the therapeutic classification is the least systematic for use that is made of drugs is after all more or less accidental rather than intrinsic A certain amount of "lack of-balance" in the treatment of the various topics becomes apparent, as, for instance, the large amount of space given to digitalis and the somewhat scanty discussion of some of the heavy metals Under the heading of Cathartics a very brief discussion of the causes of constipation is introduced, which is probably not only unnecessary but even undesirable in a book of this character Nevertheless Bastedo has given us a valuable text book which it seems is destined to make a place of its own in this field

PROTEIN SPLIT PRODUCTS IN RELATION TO IMMUNITY AND DISEASE By Victor C Vaughan Victor C Vaughan, Jr, and J Walter Vaughan Cloth, price, $3 00, one volume, total pp 474 Philadelphia Lea & Febiger, 1913

The book describes the work of fifteen years along a well defined line of thought Those who have followed the publications from Vaughan's laboratory during these years know what opposition many of his beliefs have encountered, so that it is with real enthusiasm that one reads the clear, simple statement here presented For it is obvious that almost all his tenets have been amply upheld by recent workers in immunity

The preface contains a general consideration of the chemical forces that work in the living cell, and the introduction deals with the subject of the protein structure of bacterial cells It is pointed out that like all other proteins, bacterial proteins can be split by chemical agents or ferments into a very poisonous portion and a non toxic residue The remnant of the book is concerned with a discussion of these split products and their relation to sensitization, immunity and disease The work advances in a most orderly and logical fashion The toxic portion is first established as a true protein by an exhaustive series of chemical analyses, and then there follows a description of its preparation and physiological action It is clear from these physiological tests that what Vaughan and his co workers saw many years ago in the behavior of animals injected with his "poisonous portion" was the now well known phenomenon of anaphylaxis The identity of the author's "poisonous portion" and Friedberger's anaphylatoxin is discussed at great length and with many varying points of view throughout much of the rest of the book The two substances seem to be identical

Chapters 8, 9 and 10 deal respectively with tubercle bacillus protein, anthrax and pneumococci Two important points are made in these chapters It is shown that the poisonous group (toxophor) of the tubercle bacillus kills, as all other toxic groups do, with the symptoms of anaphylaxis, but has no sensitizing power The non toxic cell residue however, can sensitize guinea pigs to living tubercle bacilli In experiments with anthrax and pneumococcus proof is advanced to support the hypothesis that pathogenicity does not depend on the amount of the toxic substance, but on the ability of micro organisms to live and multiply in the body, that is to convert human proteins into bacterial proteins

In Chapter 11, which occupies about one quarter of the book, the whole subject of anaphylaxis and protein sensitization is discussed The argument, following a short historical sketch of the knowledge of anaphylaxis, is in presentation of the author's theory of the mechanism of anaphylaxis Contrary to the generally accepted view that a mixed protein like blood serum contains one protein which sensitizes and another which is toxic, he believes that the sensitizing and toxic proteins are groups in the same molecule The process of sensitization confers the power on certain body cells of splitting such a protein molecule and liberating the toxic portion

The chapter on protein fevers is most interesting and the protocols and charts quite conclusive The last chapter, dealing with the phenomenon of infection, is likewise most suggestive and instructive Here, as elsewhere in the book, it is strongly urged that vaccine therapy has great dangers as well as power to help Consequently the author seeks the soluble protein sensitizer free from its toxic group The book is so full of ideas and of sound experimental observation that a full discussion would require much more space It is an exceptionally good piece of work, suggestive and stimulating, and the record of a most thoughtful and purposeful effort to demonstrate the obscure relationships of the protein poisons and infectious diseases

A CORRECTION

NEW YORK, May 25, 1914

To the Editor, Archives of Internal Medicine —In the article printed in your valuable journal for March, 1914, on Ulcus Ventriculi, by myself and Dr I W Held, I find the quotation from the article of J Kauffman entitled, "Mangel an Magen-Schleim," from the *Archiv fur Verdauungs-Krankheiten,* 1907, p 616, to be incorrect The first sentence reads that in hyperacidity the mucus in the stomach is increased J Kauffman found that in cases in which there is a diminution in the normal mucus of the stomach there is pain simulating hyperacidity, although the acidity is normal On that he bases his assertion that silver nitrate is beneficial in cases of irritation of the stomach in which the mucus is diminished M H GROSS

40 East Forty-First Street

A CORRECTION

Dr Edward Perkins Carter wishes to make correction in the figures as they appeared in the manuscript of his paper on "Clinical Observations on Defective Conduction in the Branches of the Auriculoventricular Bundle," in the May, 1914, number of the ARCHIVES, at page 801, as follows Page 809, line 26, for "0 13 and 0 18" read 0 12 and 0 20, p 809, line 27, for "0 18" read 0 20

INDEX TO VOLUME XIII

PAGE

Adams Stokes syndrome, three cases of, with histological findings, Adele Oppenheimer and B S Oppenheimer . 957
Addis, Thomas Urobilin its clinical significance 235
Adrenals, the alleged influence of, on diabetic metabolism, animal calorimetry Paper VIII, Graham Lusk and J A Riche 673
Agnew, J Howard Comparative study of phenolsulphonephthalein elimination and the incoagulable nitrogen of the blood in cardiorenal diseases 485
Albuminuria, orthostatic, a study of the blood pressure in children showing, Murray H Bass and H Wessler 39
Alveolar air, a comparison of methods of obtaining, Walter M Boothby and Francis W Peabody 497
Amebas, the classification of, with observation on morphology and life cycle of entamoeba coli, craigia hominis and wahlkampfia lobospinosa, Chas F Craig 737
Amylase in the urine, a clinical study of, with special reference to the phenolsulphonephthalein test, H Rawle Geyelin 96
Atophan and lithium, the influence of, on uric acid excretion of a gouty patient, Amy L Daniels 480
Atropin, a study of the action of, on the eosinophil cells of the blood, W W Herrick 794
Auricular fibrillation, paroxysmal, G Canby Robinson 298
Auriculoventricular bundle, clinical observations of defective conduction in the branches of, a report of twenty-two cases in which aberrant beats were obtained, Edward Perkins Carter 803
Auriculoventricular bundle of His, fatty infiltration (lipomatosis) of, with sudden unexpected death, Frank Nuzum 640

Baldwin, Edward R Experimental studies on the blood serum of cows immunized against tuberculosis 682
Barach, Joseph H Blood pressures, their relation to each other and to physical efficiency 648
Bartlett, Frank K Multiple primary malignant tumors, with a report of two cases in dogs 624
Bass, Murray H A study of the blood-pressure in children showing orthostatic albuminuria 39
Bastedo, Walter A Materia medica 987
Bechtel, R E A case of systemic blastomycosis with necropsy 609
Beekman, Fenwick An experimental study of intestinal obstruction 701
Blastomycosis, systemic, with necropsy, a case of, R E Bechtel and E R LeCount 609
T H Boughton and S N Clark 594
T H Boughton and A M Stober 599
T Churchill and A M Stober 568
J S Eisentaedt and T H Boughton 617
H Jackson 607
R A Krost, A M Stober and M J Moes 557
M Lewison and H Jackson 575
F B Riley and E R LeCount 614
Philip Frank Shaffner 621
A M Stober 509
H J Myers and A M Stober 585

PAGE
Blood, a study of the action of atropin on the eosinophil cells of, W W Herrick 794
Blood-flow in the hands in diseases of the heart Study X, G N Stewart 1
Blood flow in the hands and feet in certain diseased conditions of the vessels or of their venous mechanism Study XI, G N Stewart 177
Blood in chronic vascular nephritis (arteriosclerosis) as influenced by the level of protein metabolism, the non protein nitrogenous constituents of, Otto Folin, W Denis and Malcolm Seymour 224
Blood-pressure in children showing orthostatic albuminuria, a study of, Murray H Bass and H Wessler 39
Blood pressure sounds, interpretation of the auscultation, D R Hooker and J D Southworth 384
Blood-pressures, their relation to each other and to physical efficiency, Jos J Barach and W L Marks 618
Blood serum of cows immunized against tuberculosis, experimental studies of, sensitization of living tubercle bacilli, Edward R Baldwin 682
Blood, the coagulation of normal human An experimental study, Roger I Lee and Beth Vincent 398
Blood transfusion, experiments to develop a more widely useful method of, Henry S Satterlee and Ransom S Hooker 51
Boggs, Thomas R The intrameningeal treatment of tabes and cerebrospinal syphilis 970
Book Reviews Materia medica, by Walter A Bastedo 987
Organic and functional diseases, by M Allen Starr 507
Protein split products in relation to immunity and disease, by Victor C Vaughan, Victor C Vaughan, Jr, and J Walter Vaughan 987
Boothby, Walter M A comparison of methods of obtaining alveolar air 497
Boughton, T H Blastomycosis 594, 599, 617
Broders, Albert Compton Chronic gastric ulcer and its relation to gastric carcinoma Review of 684 specimens 208

Calorimetry, animal, Paper VIII The alleged influence of the adrenals on diabetic metabolism, Graham Lusk and J A Riche 673
Cardiac displacements in pulmonary tuberculosis, the mechanism of, Maurice Fishberg 656
Cardiac remedies, the comparative value of, Theodore C Janeway 361
Cardiorenal disturbances, comparative study of phenolsulphonephthalein elimination and the incoagulable nitrogen of the blood in, J Howard Agnew 485
Carter, Edward Perkins Clinical observations of defective conduction in the branches of the auriculoventricular bundle, a report of twenty-two cases in which aberrant beats were obtained 803
Cerebrospinal syphilis, the intrameningeal treatment of tabes and, Thomas R Boggs and R R Snowden 970
Churchill, T Systemic blastomycosis 568
Circulation in man X The blood-flow in the hands in diseases of the heart G N Stewart 1
Circulation in man, studies on the Study XI The blood flow in the hands and feet in certain diseased conditions of the vessels or of their venous mechanism, G N Stewart 177
Clark, S N A case of blastomycosis 594
Connor, Lewis A A pulmonary attack simulating primary lobar pneumonia caused by pulmonary embolism and infarction from a latent venous thrombosis 349
Craig, Chas F The classification of amebas, with observations on morphology and life cycle of entamoeba coli, craigia hominis and vahlkampfia lobospinosa 737

PAGE

Craig, Charles F The differential diagnosis of the intestinal entamebas of man . 917

Craigia hominis, entamoeba coli, and vahlkampfia lobospinosa, with observations on morphology and life cycle of, the classification of amebas, Chas F Craig 737

Dementia praecox, metabolism in, Ellison L Ross 889

Denis, W The non-protein nitrogenous constituents of the blood in chronic vascular nephritis (arteriosclerosis) as influenced by the level of protein metabolism 224

Digestive ferments, the mutual action of certain, J H Long and G W Muhleman 314

Eisentaedt J S A case of systemic blastomycosis 617

Emerson, Haven Status lymphaticus in adults, its clinical diagnosis and importance 169

Entamoeba coli, craigia hominis and vahlkampfia lobospinosa, with observations on morphology and life cycle of, the classification of amebas, Chas F Craig 737

Entamebas of man, intestinal, the differential diagnosis of, Charles F Craig 917

Fatty infiltration (lipomatosis) of the auriculoventricular bundle of His, with sudden unexpected death, Frank Nuzum 640

Field, Cyrus W A method developed for obtaining a standard Wassermann antigen 790

Fishberg, Maurice The mechanism of cardiac displacements in pulmonary tuberculosis 656

Fitz, R Study XXI The immediate effect of repeated doses of theobromin sodium salicylate and theocin on renal function in acute experimental nephritis 945

Folin, Otto The non protein nitrogenous constituents of the blood in chronic vascular nephritis (arteriosclerosis) as influenced by the level of protein metabolism 824

Force, J N A skin reaction indicative of immunity against typhoid fever Studies in typhoid immunization III 471

Gay, Frederick P A skin reaction indicative of immunity against typhoid fever Studies in typhoid immunization III 471

Gastric ulcer, chronic, and its relation to gastric carcinoma Review of 684 specimens, William Carpenter MacCarthy and Albert Compton Broders 208

German measles (rubella) An experimental study, Alfred F Hess 913

Geyelin, Rawle H A clinical study of the amylase in the urine, with special reference to the phenolsulphonephthalein test 96

Gonococcus complement fixation test and analysis of results from its use B A Thomas and Robert Ivy 143

Granat, Selma The urea content of the spinal fluid with special reference to its diagnostic and prognostic significance, a series of 97 cases 131

Gross, M H Ulcus ventriculi 426

Hartwell, John A An experimental study of intestinal obstruction 701

Heart block with Adams-Stokes syndrome, a pathological study of two cases of, Edward B Krumhaar 390

Heat, the effect of, on blood-pressure, L H Newburgh and C H Lawrence 287

Held, I W Ulcus ventriculi 426

Hemophilia, thrombosis and purpura, the conditions of the blood in, W H Howell 76

Herrick, W W A study of the action of atropin on the eosinophil cells of the blood 794

Hess, Alfred F German measles (rubella) An experimental study 913

v Hess, C L Hexamethylenamin 853

PAGE
Hexamethylenamin, Hugh McGuigan and C L v Hess 853
Hexamethylenamin, the value of, as an internal antiseptic in other fluids of the body than urine, Frank Hinman 841
Hinman, Frank The value of hexamethylenamin as an internal antiseptic in other fluids of the body than urine 841
Hooker, D R Interpretation of the auscultation blood pressure sounds 384
Hooker, Ransom S Experiments to develop a more widely useful method of blood transfusion 51
Houget, J P An experimental study of intestinal obstruction 701
Howell, W H The condition of the blood in hemophilia, thrombosis and purpura 76
Hypercholesterinemia, clinical study of, Harry B Schmidt 121

Immunity, some relations of the brain and of the olfactory apparatus to the processes of, Henry Sewall 856
Intestinal obstruction, an experimental study of, John A Hartwell, J P Houget and Fenwick Beekman 701
Ivy, Robert The gonococcus complement-fixation test and analysis of results from its use 143

Jackson, H A case of systemic blastomycosis 575, 607
Janeway, Theo C The comparative value of cardiac remedies 361

Krost, R A A case of systemic blastomycosis 557
Krumbhaar, Edward B A pathological study of two cases of heart-block with Adams Stokes syndrome 390
Krumwiede, Charles Attempts to produce scarlatina in monkeys 909

Lawrence, C H The effect of heat on blood-pressure 287
Lewison, M A case of systemic blastomycosis 575
LeCount, E R A case of systemic blastomycosis with necropsy 609
LeCount, E R A case of systemic blastomycosis 614
Lee, Robert I The coagulation of normal human blood An experimental study 398
Lithium and atophan, the influence of, on the uric acid excretion of a gouty patient, Amy L Daniels 480
Long, J H The mutual action of certain digestive ferments 314
Lusk, Graham Animal calorimetry Paper VIII The alleged influence of the adrenals on diabetic metabolism 673

MacCarty, William Carpenter Chronic gastric ulcer and its relation to gastric carcinoma Review of 684 specimens 208
Marks, W L Blood pressures, their relation to each other and to physical efficiency 648
Marshall, E K, Jr A comparison of the excretory power of the skin with that of the kidney through a study of human sweat 159
McGuigan, Hugh Hexamethylenamin 853
Mesonephros, the physiologic degeneration of, with a consideration of antagonistic action of organs and of functional mutation with morphological persistence, Horst Oertel 770
Metabolism in dementia praecox, Ellison L Ross 889
Moes, M J A case of systemic blastomycosis 557
Muhleman, M S The mutual action of certain digestive ferments 314
Myers, H J A case of systemic blastomycosis 585

Nephritis (arteriosclerosis), the non protein nitrogenous constituents of the blood in chronic vascular, as influenced by the level of protein metabolism, Otto Folin, W Denis and Malcolm Seymour 224

PAGE

Nephritis, the immediate effect of repeated doses of theobromin sodium salicylate and theocin on renal function in acute experimental Study XXI, R Fitz 945
Newburgh, L H The effect of heat on blood-pressure 287
Nicholl, Matthias, Jr Attempts to produce scarlatina in monkeys 909
Nuzum, Frank Fatty infiltration (lipomatosis) of the auriculoventricular bundle of His, with sudden unexpected death 640

Oertel, Horst The physiologic degeneration of the mesonephros, with a consideration of antagonistic action of organs and of functional mutation with morphological persistence 770
Oppenheimer, Adele Three cases of Adams Stokes syndrome with histological findings 957
Oppenheimer, B S Three cases of Adams Stokes syndrome with histological findings 957

Peabody, Francis W A comparison of methods of obtaining alveolar air 497
Phenolsulphonephthalein elimination and the incoagulable nitrogen of the blood in cardiorenal diseases, J Howard Agnew 485
Phenolsulphonephthalein test, a clinical study of amylase in the urine with special reference to the, H Rawle Geyelin 96
Plaggemeyer, H W A comparison of the excretory power of the skin with that of the kidney through a study of human sweat 159
Pneumonia treated with antipneumococcus serum, twenty-three cases of, William R Williams 978
Pratt, Josephine S Attempts to produce scarlatina in monkeys 909
Pulmonary attack simulating primary lobar pneumonia, caused by pulmonary embolism and infarction from a latent venous thrombosis, Lewis A Connor 349
Pulmonary embolism and infarction from a latent venous thrombosis, a pulmonary attack simulating primary lobar pneumonia caused by, Lewis A Connor 349

Riche, J A Animal calorimetry Paper VIII The alleged influence of the adrenals on diabetic metabolism 673
Riley, F B A case of systemic blastomycosis 614
Robinson, G Canby Paroxysmal auricular fibrillation 298
Ross, Ellison L Metabolism in dementia praecox 889
Rubella, German measles, an experimental study, Alfred F Hess 913

Satterlee, Henry S Experiments to develop a more widely useful method of blood transfusion 51
Scarlatina in monkeys, attempts to produce, Charles Krumwiede, Jr, Matthias Nicoll, Jr and Josephine S Pratt 909
Schmidt, Harry B The clinical study of hypercholesterinemia 121
Sewall, Henry Some relations of the brain and of the olfactory apparatus to the processes of immunity 856
Seymour, Malcolm The non protein nitrogenous constituents of the blood in chronic vascular nephritis (arteriosclerosis) as influenced by the level of protein metabolism 224
Skin, a comparison of the excretory power of, with that of the kidney through a study of human sweat, H W Plaggemeyer and E K Marshall, Jr 159
Snowden, R R The intrameningeal treatment of tabes and cerebrospinal syphilis 970
Soper, Willard E The urea content of the spinal fluid with special reference to its diagnostic and prognostic significance A series of ninety-seven cases 131
Southworth, J D Interpretation of the auscultation blood-pressure sounds 384
Starr, M Allen Book review Organic and function diseases 507

PAGE

Status lymphaticus in adults, its clinical diagnosis and importance, Haven Emerson 169

Stewart, G N Studies on the circulation in man X The blood-flow in the hands in diseases of the heart 1

Stewart, G N Studies on the circulation in man XI The blood flow in the hands and feet in certain diseased conditions of the vessels or of their venous mechanism 177

Stober, A M A case of systemic blastomycosis 509, 557, 568, 585, 599

Stober, A M Systemic blastomycosis A report of its pathological, bacteriological and clinical features 509

Sweat, human, a comparison of the excretory power of the skin with that of the kidney through a study of, H W Plaggemeyer and E K Marshall, Jr 159

Tabes and cerebrospinal syphilis, the intrameningeal treatment of Thomas R Boggs and R R Snowden 970

Theobromin sodium salicylate and theocin, the immediate effect of repeated doses of Study XXI On renal function in acute experimental nephritis R Fitz 945

Theocin and theobromin sodium salicylate, immediate effect of repeated doses on renal function in acute experimental nephritis R Fitz 945

Thompson, Loyd The titration of Wassermann reagents 904

Thomas, B A The gonococcus complement fixation test and analysis of results from its use 143

Tuberculosis, experimental studies on the blood serum of cows immunized against, sensitization of living tubercle bacilli Edward R Baldwin 682

Tuberculosis, pulmonary, the mechanism of cardiac displacements in Maurice Fishberg 656

Tumors, multiple primary malignant With a report of two cases in dogs Frank K Bartlett 624

Typhoid immunization, a skin reaction indicative of immunity against typhoid fever, studies in, III Frederick P Gay and John N Force 471

Ulcus ventriculi M H Gross and I W Held 426

Urea content of the spinal fluid with special reference to its diagnostic and prognostic significance A series of ninety-seven cases Willard E Soper and Selma Granat 131

Uric acid excretion of a gouty patient, the influence of lithium and atophan on the uric acid secretion Amy L Daniels 480

Urobilin Its clinical significance Ray Lyman Wilbur and Thomas Addis 235

Vahlkampfia lobospinosa, entamoebi coli, and craigia hominis, with observations on morphology and life cycle of, the classification of amebas 737

Vaughan, V C, V C, Jr and J W Protein split products in relation to immunity and disease 987

Vincent, Beth The coagulation of normal human blood An experimental study 398

Wassermann antigen, a method developed for obtaining a standard Cyrus W Field 790

Wassermann reagents, the titration of Loyd Thompson 904

Wessler, M A study of the blood-pressure in children showing orthostatic albuminuria 39

Wilbur, Ray Lyman Urobilin its clinical significance 235

Williams, William R Twenty three cases of pneumonia treated with antipneumococcus serum 978

CONTENTS OF VOLUME XIII

OCTOBER, 1913 NUMBER 4

OCTOBER 1913 No 4

Atypical Typhoid Infection W T Cummins, M D, and P K Brown, M D, San Francisco

Absorption of Protein Without Digestion Eleanor Van Ness Van Alstyne, New York

The Cholesterol and Cholesterol-Ester Content of the Blood in Xanthoma Tuberosum Jacob Rosenbloom, M D, Ph D, Pittsburgh, Pa

The Relation of Fatigue to Paralysis Localization in Plumbism Ralph R Mellon, M D, Ann Arbor, Mich

On the Reinspiration of Expired Air Thomas R Crowder, M D, Chicago

Functional Tests of the Kidney in Uremia Nellis B Foster, M D, New York

Blood-Pressure Studies in Tuberculosis at a High Altitude Report of Six Hundred Cases LeRoy S Peters, M D, and E S Bullock, M D, Silver City, N M

"Auricular Flutter" with Report of Two Cases Frank Taylor Fulton, M D, Providence, R I

NOVEMBER, 1913 NUMBER 5

The Cause of the Specific Dynamic Action of Protein Graham Lusk, New York

Sources of Error in the Use of the Stomach-Tube [illegible] Preliminary Report Tom [illegible] M D, and Walter J Dor [illegible]

Thrombosis Ludwig Aschoff, Freiburg, i Br, Germany

Studies of Blood-Pressure in States of Excitement and Depression M C Hawley, M D, Watertown, Ill

Age Incidence in Carcinoma Carl Vernon Weller, M D, Ann Arbor, Mich

Heredity with Reference to Carcinoma Aldred Scott Warthin, M D, Ann Arbor, Mich

A Clinical Study of Vagotonia Arthur H Hopkins, M D, Philadelphia

Experiences with Prophylactic Typhoid Vaccination Its Effect on Menstruation Albert R Lamb M D, New York

Hexamethylenamin The Liberation of Formaldehyd and the Antiseptic Efficiency under Different Chemical and Biological Conditions Paul J Hanzlik, M D, and R J Collins, A B, Cleveland

DECEMBER, 1913 NUMBER 6

The "Typhoid Carrier" State in Rabbits as a Method of [illegible]

and Edith [illegible] Cal

Agglutinability of Blood and Agar Strains of the Typhoid Bacillus Studies in Typhoid Immunization II Frederick P Gay, M D, and Edith J Claypole, M D, Berkeley, Cal

The Examination for Diagnostic Purposes of the Enzyme Activity of Duodenal Contents Arthur F Chace, M D, and Victor C Myers, M A, New York

II Hemorrhagic Disease Antithrombin and Prothrombin Factors G H Whipple, M D Baltimore, Md

Practical Studies on the So Called Syphilis "Antigens," with Special Reference to [illegible] Extracts J A Kolmer, [illegible]baugh, M D, A J Cassel- [illegible] W W Williams, M D

Studies on the Circulation in Man IX The Blood Flow in the Hands (and Feet) in Cases in which Obvious Anatomical Differences Exist G N Stewart, M D, Cleveland, O

Blood Transfusion and Regeneration in Pernicious Anemia Karl M Vogel M D, and U F McCurdy, M D, New York

[illegible] of the Contracted Kidney Freiburg i Br

[illegible]cid of Blood and Urine, with Special Reference to the Influence of Atophan James S McLester, M D, Birmingham, Ala

Some Forms of Urinary Nitrogen Affected by the Administration of Desiccated Thyroid to Dementia Praecox Patients Ellison L Ross, Ph D, Kankakee, Ill

Morphology of the Blood in Epidemic Poliomyelitis Joseph H Baiach, M D, Pittsburgh, Pa

A Clinical Study of Hypertensive Cardiovascular Disease Theodore C Janeway, M D, New York

Book Reviews